

BIOLOGICAL REVIEWS

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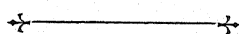
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GASTRIC DIGESTION IN THE LOWER VERTEBRATES

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(Received 20 February 1941)

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I. INTRODUCTION

ALTHOUGH the literature on the digestive system of the lower vertebrates is by now extensive, the elucidation of the physiological processes involved falls far short of that which has been achieved for the mammals. Apart from the obvious reasons which have led to this latter group attracting more attention, the deficiency seems to result also in part from an inclination of the physiologist to approach the lower forms as though they were simplified mammals, while the zoologist has not always appreciated the wider physiological significance of his own special problems. Further progress in this field thus seems to demand a synthesis of these viewpoints, and it is the aim of the present article, in addition to reviewing the recent literature, to indicate some paths along which this might be achieved within the field of gastric physiology, although the evidence is still too incomplete to admit of more than tentative generalization. The groups dealt with are the fishes and Amphibia, the mammals being introduced only in order to illustrate the type of problem involved. Reference should be made to Vonk (1937,¹ 1939) for a more detailed study of the digestive enzymes, and to Yonge (1931, 1937¹) for reviews of recent work in digestive physiology with special reference to the invertebrates.

Since it will not be possible to discuss morphological and histological problems, it will be convenient to summarize briefly certain facts which must be borne in mind in interpreting the physiological data. The stomach of fish (Jakobshagen, 1913; Plenck, 1932; Pernkopf & Lehner, 1937) is commonly flexed into a proximal

¹ *Biological Reviews.*

(descending) and distal (ascending) limb, which should be termed the 'corpus' and the 'pyloric region' respectively, in order to avoid the false analogy with the mammals involved in the term 'cardiac region' which is often given to the former; from the angle a caecum may extend back, especially in the Actinopterygii. The shape and proportions of these parts are so variable that much caution must be exercised in generalizing as to functional differences between them from observations based on only a few types. The lining epithelium commonly resembles that of mammals in being composed of slender mucoid cells which presumably produce, as in the latter group, the mucus which protects the surface of the stomach from injury (Babkin & Komarov, 1932). Certain fish, including *Torpedo* and the cyprinodont *Belonesox* (Helly, 1905; Baecker, 1934), possess an additional type of secretory cell, resembling pancreatic zymogen cells or the Paneth cells of the mammalian intestine; these, together with the presence of cilia in *Acipenser*, *Amia* and *Lepidosteus* (Macallum, 1886; Hopkins, 1895; Rogosina, 1930), may perhaps represent the retention of primitive characters. The gastric glands of fish show a more primitive level of organization than those of mammals, for pepsinogen and acid are generally considered to be produced by the same cell instead of by separate chief and parietal cells, although the evidence for this is very incomplete; moreover, while the glands which contain these cells in the mammal (the 'proper gastric glands') have a neck region occupied by a special type of 'mucoid neck cell' differing from the surface cells, no such cell is found in fish, with certain possible exceptions amongst the more highly differentiated Teleostei (Bensley, 1932; Plenck, 1932). The glands ('chief glands') tend to be confined to the corpus, so-called 'pyloric glands', as pointed out by Yung (1899), being often merely crypts of the surface epithelium, and not comparable with the true pyloric (or cardiac) glands of mammals, which are composed of cells resembling the mucoid neck cells of the proper gastric glands. The chief glands may, however, extend into a part of the pyloric region, the extent to which they do so probably reflecting the course of embryonic differentiation, which appears to begin in the middle of the stomach and to proceed forwards and backwards (Egounoff, 1907).

In Amphibia the stomach is lined, as in fish, with slender mucus-secreting cells (Tschassownikow, 1927). In form it is typically spindle-shaped, but corpus and pyloric regions can be distinguished histologically, the former, occupying from four-fifths (*Bufo*; Béguin, 1904) to three-quarters (*Necturus*; Kingsbury, 1894) of the total length, possessing the chief glands, which show an advance on the condition in fish in the presence of distinctive mucoid neck cells. In certain forms, as in *Rana temporaria*, *Triton* and *Bufo* (Langley, 1881; Bensley, 1898; Lim, 1922), the pyloric region has characteristic glands which are formed of these same mucoid cells without the zymogen cells, and which thus recall the pyloric glands of mammals (but see p. 6). Oesophageal glands are common in Amphibia (Bensley, 1900; Machan, 1935); fundamentally, they form a continuous series with the gastric chief glands, with a tendency for arrested development in some Urodeles, while in Anura the anterior members of this series become predominantly pepsinogen-secreting and the posterior members predominantly acid-secreting. Bensley has commented

upon the parallelism between this trend and the evolution of chief and parietal cells in the mammalian glands.

II. EVOLUTIONARY CONSIDERATIONS

It is important to realize that peptic digestion, which has been convincingly demonstrated in animals only in the Chordata (Oppenheimer, 1939), is absent from the lower members of that group, the protease in *Glossobalanus* (Barrington, 1940), *Tethyum* (Berrill, 1929) and *Amphioxus* (Barrington, 1937) appearing to be of the tryptic type, although Krüger (1929) has argued that in *Ciona* it resembles yeast protease rather than trypsin. As regards the Cyclostomata, peptic digestion is lacking in the ammocoete larva (Barrington, 1936), and probably also in the adult lamprey, since the cells associated with the production of the larval trypsin are also present in that stage, while there is no histological differentiation of a stomach (personal observations). Comparative anatomists would probably agree that this absence of a stomach from the Cyclostomata is a primitive rather than a secondary character, although the current interpretation of these animals as degenerate survivors of a group in which, for example, bone was originally present makes this conclusion less obvious than it might once have seemed, especially since the stomach has probably been secondarily lost in a number of fish (see later). In contrast to the latter, however, the alimentary system of the Cyclostomata shows a decidedly primitive feature in the organization of the pancreas (Barrington, 1936), and, perhaps more significant, the feeding habits throughout the group resemble those of the lower Chordata in being essentially microphagous. This is most clearly seen in the ammocoete larva (Newth, 1930), but many of the extinct forms appear to have been suctorial bottom feeders, and both this and the semi-parasitic habits of living adult forms are easily derived from microphagy.

If, then, it be assumed that the continued absence of a stomach from the Enteropneusta to the Cyclostomata is correlated with a general uniformity in feeding habits, it becomes possible to view the development of that organ in vertebrates as being determined by a fundamental change in those habits, for its appearance in fish is associated with the introduction of macrophagous feeding effected through the agency of jaws, and it can be visualized as developing as a chamber in which the bulky food could be temporarily stored and prepared for subsequent digestion. With this once established, the further evolution of the power of secreting acid would have had several advantages. This acid is known to have a toxic effect on live prey (Dorris, 1935), and is important in many fish for its decalcification of ingested exoskeletons (Sullivan, 1905), particularly since vertebrates do not secrete a chitinase. The gastric juice of mammals is further known to have bactericidal action, destroying *Bacillus coli* and streptococci (Best and Taylor, 1940), and this may be supposed to apply also to the lower vertebrates. Johnson (1904) found *B. coli* in forty-seven out of sixty-seven fish examined by him, and stated that it occurred in the stomachs of twenty-four and in the intestine of forty-one; however, three species of the fish studied by him were cyprinoids, which would

probably not have possessed a true stomach at all (see below, § VIII), and if his figures are revised accordingly it appears that *B. coli* occurred in the intestine of forty-one fish but in the stomach of only nine. This gives some indication of antiseptic properties which would probably be of advantage in poikilotherms where the food may remain for several days in the stomach. The production of pepsin would presumably have followed that of the acid; their simultaneous appearance would have involved a more complex physiological evolution, particularly if it be true that in the lower forms they are both secreted by the same cell, while pepsin could scarcely have appeared first since it requires the presence of acid for its action. This suggested course of evolution of the stomach is somewhat hypothetical, but it serves to emphasize the important fact that it is an organ which has developed gradually within the vertebrates; with this in mind it can be seen that gastric digestion in fish will represent a relatively early stage in the evolution of that function, and may therefore be expected to differ from the high level of specialization attained in the mammals.

III. ENZYMES

The production of pepsin in the stomach of fish and Amphibia is thoroughly well established. To mention some of the more recent work, it has been identified in mucosa extracts of various selachians and teleosts by Bodansky & Rose (1922), in the gastric contents of *Scyllium canicula* and other forms by Dobreff (1927), in the mucosa of *S. catulus* by Polimanti (1912), and in the filtered extracts of the mucosa of *Raja* and *Torpedo* by Weinland (1901). Almy (1926) and Battle (1935) have identified pepsin in extracts of the mucosa of the herring, Bayliss (1935) in extracts and in the gastric juice of *Pleuronectes platessa*, and Polimanti (1912) in the mucosa of *Box* and the conger. The classic work of Langley (1881) on the Amphibia, in which he demonstrated the parallelism between pepsin content and the abundance of the zymogen granules in *Rana*, *Triton*, *Bufo* and other forms, is too well known to require comment.

The identification of the enzyme as pepsin depends especially upon determinations of the optimum pH. Bodansky & Rose (1922) found that *Squalus* had an optimum at pH 3 for gelatine digestion. Vonk (1927), using purified extracts from the same animal, found the optimum for fibrin digestion to be pH 2.29-2.44, and later (1929) showed that the optima for the enzymes of *Squalus (Acanthias)*, *Esox*, the frog, *Testudo* and the pig approximated to pH 2, thus providing some evidence for the identity of the enzyme throughout the vertebrates. Other workers have arrived at results essentially similar, taking into consideration differences in method, substrate and enzyme preparation; for the herring the optimum has been placed at pH 2.5-2.85 (Almy, 1926), for *Pleuronectes* (Bayliss, 1935) at 1.5-2.5 (casein), for the perch (Hykes *et al.* 1934) at 1.65 (fibrin) and 1.8 (gelatine). Some authors have interested themselves in establishing how far the enzyme is adapted to the lower body temperature of these poikilotherms, but most of the results, as far as they go, offer little positive evidence for such adaptation. Kenyon (1925)

found fish pepsin more active at 37° C. than at room temperature, with about the same proportionate increase in activity in amphibians, reptiles and mammals. Almy (1926) and Battle (1935) found herring pepsin more active at 37° C. than at lower temperatures; perch pepsin has a temperature optimum at 30° C. (Hykes *et al.* 1934), and frog pepsin shows an increase in activity up to 40° C. (Müller, 1922). Riddle (1909) found that the gastric digestive power in fish and other poikilotherms was reduced by one-third in winter, but this is not necessarily a measure of increasing nutritional difficulty, for according to Scheuring (1928) the rate of metabolism, and hence the amount of food required, falls more rapidly than the rate of digestion as the temperature decreases (in *Misgurnus fossilis*). Kenyon (1925) found no support for Riddle's view that a loss of digestive power occurred in ascending from fish to reptiles. Some positive indications for the existence of minor differences between the pepsins of the pike and the dog were obtained by Rakoczy (1913), both he and Vonk (1937) finding the former to show a greater thermolability. The facts suggest a fundamental resemblance between the pepsins of the vertebrate groups, with some minor adaptations to their medium, perhaps effected by associated materials similar to that which modifies the pH optimum of mammalian gastric lipase (Willstätter & Memmen, 1924). A number of variables are, of course, involved, and Chesley (1934) has pointed out that it is meaningless to speak of optimum temperature without specifying pH, ionic concentration, digestive time and enzyme-substrate ratio.

The distribution of pepsin has been shown to correspond with that of the granular zymogen cells. Yung (1899) detected pepsin in the corpus but not in the pyloric region of *Scyllium canicula*, and Beauvalet (1933*b*) later confirmed that only the former region digested fibrin to albumoses and peptones in an acid medium. Sullivan (1905) found no digestive activity in the pyloric region of a number of selachians, and Herwerden (1908), while noting that that region gave a slight peptic activity, considered this due merely to some pepsin carried over from the corpus. Polimanti (1912) investigated the distribution of some enzymes in several fish, and found that in *S. catulus* pepsin was most abundant at the angle of the stomach, less at the upper end of the corpus, and least near the pylorus. In *Box* most of the pepsin occurred in the caecum, less in the upper corpus and least in the pyloric region. Stirling (1884) found that in both the herring and the haddock more pepsin could be extracted from the corpus than from the pyloric region. The oesophageal zymogen cells of Amphibia are known to be especially active in the production of pepsin. Far more of this enzyme can be extracted from the oesophagus of the frog than from the stomach, and the least from the pyloric region, while in *Pelobates*, *Hyla*, *Bufo* and *Triton* also the oesophagus is richer in pepsin than is the stomach (Swiecicki, 1876). Langley's precise studies (1881) showed that pepsin could be extracted from the oesophagus and stomach of *Rana temporaria*, but not from the pyloric glands, and that in *Bufo* most of the pepsin was localized in the mucosa of the hinder oesophagus and anterior stomach, with very little in the pyloric region. Pjatnitzkij (1930) obtained pepsin from both the stomach and the oesophagus of *Rana esculenta*, and Friedman (1934), employing the same species, obtained far

more pepsin from the oesophagus than from an equal area of the stomach. Dorris (1935) believed that pepsin was found in the anterior end of the stomach of the larva of *Amblystoma*, which agrees with the known course of development of the glands (Bensley, 1900).

Very little is known about the localization of the production of acid in the stomach of these lower vertebrates. That acid is secreted in the corpus of the selachian seems clear from the acidity of the contents obtained from that portion; whether it is secreted in the pyloric region is uncertain, although Herwerden's (1908) observation, that after washing the mucosa the corpus wall was acid and the pyloric neutral, suggest the possibility that it is confined to the former region. The production of acid in the frog seems to be confined to the stomach; the oesophagus produces only pepsin and mucus (Friedman, 1937), while oesophageal strips, unlike gastric ones, are unable to secrete acid in vitro (Gray *et al.* 1940). Friedman (1934) found that when the pyloric region was tied off from the corpus, mechanical stimulation created by glass beads placed in either region caused the production of acid only in the pyloric; this is surprising, for by analogy with mammals, the pyloric glands (when, as in *Rana*, they possess mucoid cells) might be expected to produce an alkaline secretion, in order to reduce the acidity of the gastric contents prior to their entry into the duodenum (Florey & Harding, 1933). Grützner (1905), moreover, found that in the stomach contents of freshly killed frogs and toads which had been fed upon bread, milk and litmus, only the anterior end gave an alkaline reaction (from the oesophageal secretion), but it seems probable that this method would hardly detect such a suggested reduction of acidity. Dorris (1935), feeding young *Amblystoma* larvae with stained enchytraeid worms, obtained some evidence that the pepsin was located at the anterior end of the stomach while the greater acidity was found at the hind end, but this form (at least in the adult) seems not to possess mucoid cells in the pyloric region (Bates, 1904).

The possibility of the secretion in the stomach of enzymes additional to pepsin has been considered by only a few authors; it is nevertheless of importance in view of the known presence in the mucosa of more than one type of secretory cell in some forms, and of the possibility of divergence in pH between corpus and pyloric region resulting from the localization of the production of acid, and of other factors which will appear below. 'Rennin' was identified in *Torpedo*, but not in *Squalus* or the sawfish, by Bodansky & Rose (1922), by Herwerden (1908) in various selachians, and by Polimanti (1912) in *Scyllium catulus*, *Box* and the conger; Dobreff (1926), on the other hand, could not identify chymosin in *Scyllium canicula* and *S. stellare*, but in any case these authors do not appear to have distinguished between true chymosin and the milk-clotting action associated with proteases in general (see Oppenheimer (1939) for a discussion of this point). Ege & Obel (1935), however, have identified a true chymosin in the cod. No chitinase has been found in selachians (Yung, 1899; Herwerden, 1908), although a chitinous exoskeleton is common in their prey, and unmodified chitin consequently occurs in the faeces. Sullivan (1907) found no amylase or lipase in corpus or pyloric extracts of selachians, but Weinland (1901) thought that there was probably an amylase in

the extracts and in the alkaline (see below, p. 8) gastric juice of *Raja*. Herwerden (1908) could not detect any digestion of glycogen after introducing it into the selachian stomach, and found no amylase in the stomach of *Mustelus*, even when the latter gave an alkaline reaction; she was unable to test Weinland's finding, as only acid-reacting *Raja* were available. Lipase tests with olive oil and egg yolk were negative, but positive results were obtained in *Raja*, *Acanthias*, *Cyclopterus* and *Gadus* with monobutyrim, the enzyme concerned being mostly in the corpus; also using monobutyrim, Polimanti (1912) identified a lipase in *Scyllium catulus*, *Box* and the conger. Beauvalet (1933*b*) found that the corpus of *Scyllium canicula* had no action on starch and olive oil, but that the pyloric region digested both in an alkaline medium. Possible indications of tryptic action have been obtained by Weinland (1901), who found that filtered mucosa extracts of *Raja* and *Torpedo* not only digested fibrin to albumoses in an acid medium, but did so also, although more slowly, in an alkaline one, and by Beauvalet (1933*b*), who showed that the pyloric region of *Scyllium canicula* digested fibrin to peptones in an alkaline medium. Additional observations on the teleosts include the identification of a weak lipase and amylase in the gastric juice of the herring (Battle, 1935), and a slight amylolytic action in the stomachs of *Pomoxis* and *Esox*; Kenyon (1925) considered the latter too weak to be of digestive significance, and Bayliss (1935) was unable to identify any gastric lipase or amylase in *Pleuronectes*. There seems to be no record of such observations on Amphibia.

The above findings seem sufficiently positive at least to justify further investigation of the properties of the gastric mucosa. Any enzymes identified in extracts may, of course, be of more than one origin; they may have been regurgitated from the intestine, they may be present in the blood (especially the amylase), or in wandering leucocytes, as in the pyloric secretion of the dog (Kestner *et al.* 1929). They may also represent intracellular enzymes located in the epithelial cells, and in this connexion it should be remembered that there is evidence that certain of the enzymes of the intestinal juice of mammals are of an intracellular nature (Wright *et al.* 1940).

IV. STOMACH CONTENTS

Sullivan (1905) found the reaction of the fasting stomach of various dogfish at Woods Hole to be 'practically' neutral, but others have obtained a marked acid reaction from such animals. The gastric juice of *Scyllium*, *Torpedo* and *Raja*, as obtained by siphoning (Weinland, 1901), is a clear fluid of which as much as 10-50 c.c. can be secured from a single starved *Scyllium*; even after several weeks starvation, the juice will turn congo paper blue, implying the presence of free acid. Weinland assumed that this acid was organic, but Herwerden (1908) and later Herwerden & Ringer (1911) showed that although some formic acid could be identified, the acidity in *S. stellare* was mainly due to hydrochloric acid. Dobreff (1926, 1927) claimed that the gastric secretion in *S. canicula* was continuous from embryonic life to death, although diminishing during starvation, and identified free

hydrochloric acid. Herwerden found that there was always an acid reaction in the stomach of *S. canicula* and *S. stellare*, *Mustelus laevis*, and *Torpedo marmorata* and *T. ocellata*, whether food was present or not. Babkin *et al.* (1935*a*) obtained gastric juice from the fasting skate (*Raja diaphanes* and *R. erinacea*), but it was small in quantity, 0.5 c.c. being obtainable only with difficulty; it was an acid, mucoid fluid, pH 3.3-3.8.

It seems clear that the acidity, and presumably the volume, of the secretion increases after the taking of food, and may reach very high values, Pilliet (1885) long ago commenting on the very high acidity of the gastric contents of selachians. Soulima (1919) fed 70-80 cm. *Scyllium catulus* with fresh or cooked sardine (40 g.), introducing the food and later removing the contents by a glass tube. The maximum acidity was attained between 48 and 72 hr. after feeding, a result which agrees with Dobreff's (1927) statement that maximum acidity (*S. canicula*, etc.) is found 'some days' after feeding. Values for total acidity in terms of hydrochloric acid are high, 1% (Sullivan, 1905) and '45 c.c. of N solution per 100 c.c. liquid contents' (Soulima, 1919) having been recorded. The acidity of human gastric juice, even as secreted, is only 0.5%, and may be lowered by various factors after secretion to 0.2% (Hawk & Bergeim, 1937).

The above facts apply mainly to sharks, for in rays, according to the work of Weinland (1900), the gastric juice may be acid or alkaline. The blood vessels of the mucosa in *Raja* possess smooth muscle sphincters which can be stimulated to close by the injection of extract of secale cornutum, and he was able to show that this condition was associated with the secretion of an alkaline juice, the reaction becoming acid when the sphincters were relaxed. He therefore claimed that the latter constituted the mechanism responsible for this alternation of acidity with alkalinity, and they certainly seem to be absent from *Scyllium* and *Torpedo*, in which forms the reaction is normally acid. It is not clear whether this sphincter mechanism is widespread amongst rays, for Herwerden (1908) found two *Raja clavata* with alkaline contents containing Crustacea and mucus, but seven others with Crustacea and fish gave an acid reaction. Other workers have noted the appearance of an alkaline fluid in their work on these animals (e.g. MacIntosh, 1935), but have assumed it to be due to contamination with sea water entering the stomach under experimental conditions.

Digestion proceeds slowly in poikilotherms, and food may remain for a considerable time in the selachian stomach, for the dogfish is estimated to require six times as long for the digestion of protein as does the mammal (van Slyke & White, 1911). The time of evacuation of a mixed meal from the human stomach ranges from 3 to 4½ hr. (Best & Taylor, 1940), but Weinland (1901) found that food remained in the stomachs of *Scyllium*, *Torpedo* and *Raja*, kept in tanks at 13-15° C., for 2, 3 or more days, and in the case of one *Scyllium* for as long as 18 days. The time of digestion will depend upon the consistency and composition of the food. Van Slyke & White (1911), feeding dogfish on the very artificial diet of chopped beef, found that within 6 hr. a considerable portion of the coagulated protein was dissolved and absorbed, and that between 6 and 12 hr. after feeding, digested and

solid protein was passing into the intestine; solution and absorption of the meal was in this case nearly complete after 3 days, but the conditions were clearly far removed from those of natural feeding. Dobreff (1927) observed that defaecation in a dogfish first occurred 5 days after a meal. In view of the possibility of secretagogues from the natural food having a stimulating effect on gastric secretion (see p. 13), it is of interest to note that during the first 2 days of digestion, uncooked sardine was 'dissolved' more quickly than was cooked in the stomach of *Scyllium catulus* (Soulima, 1919). One of the few indications of divergence of function between corpus and pyloric region is the same author's observation that the main dissolution of sardine flesh occurred in the stomach (corpus) of this animal, the contents passing by a narrow orifice into the 'pyloric intestine' (presumably the pyloric region of the stomach); when removed from the latter through a fistula they showed hardly any suspended particles.

Observations of the gastric contents of teleosts have been mainly confined to the state of the food, and little information is available on the properties of the gastric juice itself. As in the selachians, digestion commonly occupies a lengthy period, the digestion of large prey by *Esox* taking 3-5 days (Vonk, 1929). *Cottus* and *Gadus callarius* and *G. virens* (Karpevitch & Bokoff, 1937) take 5-6 days for the digestion of fish, and 3-3½ days for *Gammarus*; *Pleuronectes flesus* takes 2½ days for *Gammarus* and 2 days for molluscs. According to Pierce (1936), 25 hr. after a meal of anchovy, *Ocyurus* still retains some undigested material in its stomach. Sokolov & Chvaliova (1936) have reported that the stomach of *Gambusia* is completely emptied of a meal of *Anopheles* larvae or *Daphnia* in 3-4 hr., which would be a remarkably rapid time even allowing for the temperature of 30° C. at which the observations were made; in fact, however, this animal does not possess a stomach (see Table 1), and the figures thus apply merely to the anterior swollen end of the intestine. In *Pleuronectes platessa* (Dawes, 1930), even partial relaxation of the pyloric sphincter does not occur until 16-18 hr. after a meal of mussel, finely divided solid and fluid food reaching the intestine after 19 hr.; complete relaxation of the sphincter occurs after 40-48 hr., and the gut is cleared of food after 54-60 hr. Such times will be modified by various factors. Thus the food will pass more quickly if a second meal is taken soon after the first (Dawes), from which it would appear that the more frequently such an animal feeds, the less efficient will be its utilization of the ingested material. *Pleuronectes flesus* (Karpevitch & Bokoff, 1937) will take a second meal 14-15 hr. after a previous one, although 2-2½ days are required for complete digestion.

Herwerden (1908) found some teleosts with acid stomach reactions, but many with neutral or alkaline ones, irrespective of whether or not they contained food, and she concluded that in general the teleost stomach was much less strongly acid than that of selachians. This seems to be borne out by the experience of other workers. The stomach contents of *Esox* have a pH of 4.5-4.7 (Vonk, 1929), and Kenyon (1925) found that the pH of the stomach of various teleosts (*Lepomis*, *Esox*, *Perca*, etc.) varied from neutrality to 4, the acidity being higher when food was present. Bayliss (1935) found that the (usually empty) stomach of *Pleuronectes*

platessa often gave values between pH 5.6 and 7.6; even when food was present the acidity was not so high as might have been expected in view of the known presence of pepsin, although he showed that acid was certainly secreted into the stomach contents. Herwerden's statement, that an alkaline reaction in *Pleuronectidae* is easy to understand since the stomach is in wide communication with the intestine, seems not to be applicable to *P. platessa*, in which a well-developed pyloric sphincter and valve are present (Cole & Johnstone, 1901), but the sole is described as having a stomach opening wide into the oesophagus and intestine, with no constrictions (Valatour, 1861). In *P. flesus* the stomach is small and only slightly differentiated; after the taking of food the stomach reaction is at first pH 7.7, and even towards the end of digestion has only fallen to 5.5 (Karpevitch & Bokoff, 1937). It might be supposed that under such conditions, so far removed from the optimum pH for peptic activity, gastric digestion must be relatively incomplete, and there seems some indication that this may be so, for in this particular fish no 'grinding' occurs in the stomach, and whole food objects (*Gammarus*) pass into the intestine or even into the faeces. However, this is not a general rule, for these authors, comparing *Cottus*, *Gadus callarius* and *G. virens* with *Pleuronectes flesus*, found that in the former animals there was a large stomach and small intestine; the fasting stomach had a pH of 3, and although it became alkalinized after feeding, the acidity slowly increased again to pH 3-4, the exact conditions depending on the food (fish, *Gammarus*). Here, complete destruction of the food occurred in the stomach, and the same seems to be true of the herring, the normal food of which includes Crustacea and Chaetognatha. Battle (1935) fed copepods to this animal, which is known to gorge until the caecum is distended to several times its size when empty. Examination of the stomach contents 5-7 hr. after feeding showed that the animals at the cardiac end were undigested, those in the caecum showed minor fragmentation with some digestion of muscles and much liberation of oil globules, while the pyloric region contained finely divided portions of copepods, much of their chitin being entirely free from the muscles. In *Salmo trutta* digestion does not, perhaps, proceed so far, for Pentelow (1932) noted that the stomach contents, undigested at the cardiac end, were still identifiable in the pyloric region.

Reviewing the above rather scattered results, it seems likely enough that the teleost stomach is less acid than the selachian, and that the pH of its contents does not approximate as closely as might be expected to the optimum for pepsin. There is some reason to believe that this is compensated by a greater production of that enzyme, which was found by Vonk (1929) to be more abundant in the pike than in *Squalus*, where the acidity is much greater, and the same author has also demonstrated the importance of another factor by measuring the pH at isolated points within the stomach by means of the capillary glass electrode. The results (Vonk, 1939) show that while the pH of the general stomach contents of *Esox* is 4.5-4.7, the actual pH at the surface of a large fish lying within the stomach may range from 2.4 to 3.6, which agrees well with the optimum pH for the pepsin of the stomach contents (2.4). The importance of this surface effect is clearly shown in that where the pH at the surface of the prey is 2.6, it is found to be 2.8 at 2 mm. depth, and

4.7 at 3 mm., and this agrees with other observations of the condition of prey during the digestive process. Pierce (1936) fed *Ocyurus* and *Haemulon* kept at 24° C. with anchovy, and found that 9 hr. of gastric digestion had only effected removal of the thin outer skin and effacement of slight surface markings. Obst (1919) records finding two silver hake, one with twenty-one and the other with twenty-three herring in the stomach; those portions of the prey which lay against the stomach mucosa were markedly digested, but the remainder of the fish were firm and solid. This gorging with bulky food is no doubt of common occurrence in fish; the stomach of an 80 cm. Pacific salmon (*Oncorhynchus*) may contain eighteen small squid and several small fish (Greene, 1912 a), while in the cod (Stirling, 1884) there may be found fish like haddock in every stage of disintegration, and frequently also Crustacea, molluscs, holothurians and *Aphrodite*. The importance of taking this factor into account in interpreting the results of laboratory experiments on extracts or on fluid gastric contents is sufficiently obvious. Whether or not the surface effect is operative throughout the period of gastric digestion depends on circumstances such as the nature of the food. According to Vonk (1939) the conditions in the perch resemble at the beginning of gastric digestion those in the pike, but later, when part of the food has left the stomach, the whole contents approximate in pH to the optimum for pepsin. Presumably this is a result of the perch feeding on smaller organisms, which are more readily digested, but this animal's diet is known to vary with its size (Allen, 1935), and it would be of interest to know whether this affects the course of gastric digestion; according to Vonk, the perch resembles *Squalus* in producing relatively much less pepsin than the pike. The latter animal is recorded as feeding with some frequency on nymphs of *Ephemera danica* when these are available (Allen, 1939), but at other times it seems to select perch in preference to the smaller *Gasterosteus* and *Phoxinus*. In contrast to this relative constancy of diet, the trout is a carnivore which will feed upon virtually whatever invertebrates are available in its stream, with a tendency upon the part of the largest to take fish (Pentelow, 1932; Slack, 1934; Allen, 1938). How far such characteristics of diet are determined by the properties of the stomach, or how far the latter are modifiable according to the type of food eaten, are questions which still await an answer.

A few observations are available on the gastric juice of the frog, which was obtained by Smirnoff (1918) from animals in which the cardiac and pyloric ends of the stomach had been tied off after filling it with pieces of cork (see p. 14 for the effect of mechanical stimulation). After some time, 0.5 c.c. of clear, opalescent juice with many white clumps was obtainable; it was strongly acid to methyl orange, and had a strong digestive activity. He also (1922) prepared animals with gastric fistulae, and obtained a slimy secretion with an acid reaction and with a high concentration of pepsin. Pjatzitzkij (1930), using Smirnoff's first method, obtained from *Rana esculenta* a gastric juice with a pH of 1.52-3.9, well adapted, therefore, for the action of pepsin, but differing from the gastric juice of the dog, which was found to have a pH of 0.93-0.98; this difference is perhaps due to the very mucous nature of the frog's secretion, for both authors state that most of the

acid is present in combined form. Delrue (1930) found the gastric contents of the frog to range from pH 1.90 to 4.92. The pH of human fistula juice, it may be noted, is 0.9-1.5, and of the stomach contents during digestion of a mixed meal 1.3-2.5 (Best & Taylor, 1940). Dorris (1935), feeding early *Amblystoma* larvae with stained encytraeids, estimated the pH of the stomach to lie between 1.4 and 4; an acid reaction was obtained in the base of the stomach within 20-30 min., but not at the cardiac end until after 12 or more hours. Vonk (1939) found that 46 hr. after a meal of chopped meat the pH of the frog's stomach, measured by the capillary glass electrode, ranged from 2.20 to 3.75, the latter at the anterior end; this animal resembles the perch, according to his analysis, in that at the beginning of digestion only the surface layer of the prey, which is here small in proportion to the size of the stomach, has a pH suitable for peptic action, while later the contents as a whole approximate to that figure.

As with fish, food remains for a long time in the amphibian (frog's) stomach. Langley (1881) found that a frog required 24 hr. to remove a small worm from its stomach, while *Rana catesbiana* requires 48-68 hr. for the removal of a small frog (Patterson, 1933). The digestion time varies according to the quantity of food present (Frost, 1932), and may extend to 3 days or longer. When the stomach is crowded with food, digestion is not complete, and whole insects or large parts of them may be discharged with the faeces. Parts of a beetle have been recovered from a frog 17 days after feeding. One of the questions suggested by this lengthy digestion period is whether the animal maintains a constant output of enzyme. Soulima (1919) found that the peptic activity in the corpus of *Scyllium catulus* hardly varied throughout digestion, but observations of Langley (1881) suggest that in the Amphibia some variation may occur, for he found that both in the oesophageal and in the gastric glands the zymogen content, reduced in the early stages of digestion, began to return to the normal 'resting condition' some time before the evacuation of the stomach. As is to be expected, digestion and the mobility of the alimentary tract are increased by a rise in temperature (*Rana esculenta*); digestion is said to cease at and below 6° C. (Turbin, 1925), although it is known that some enzymic activity can proceed at 0° in vitro (Smith, 1938).

V. THE CONTROL OF GASTRIC SECRETION

The control of gastric secretion in mammals is a highly complex process which will be summarized here rather dogmatically for purposes of comparison with the lower forms; for a full discussion, with bibliography, of the many problems involved, reference may be made to Best & Taylor (1940). Prior to the entry of food into the stomach, there is a psychic or cephalic phase of secretion, mediated through the vagus nerve, stimulation being effected by, for example, the sight or smell of food. With the arrival of food in the stomach there sets in the second, or gastric, phase, which is essentially humoral in nature, and is therefore operative even when the stomach is separated from the central nervous system. To some extent the mechanical stimulation of the food (particularly any distending force) and the

presence of water contribute to this phase, but the effect is mainly due to the presence of meat extractives and the products of proteolytic digestion (proteoses and peptones); the action of these substances on the gastric mucosa results in the liberation from the pyloric region into the blood stream of a secretagogue which stimulates the gastric glands. Since the pyloric region is rich in histamine, and since this substance is known to stimulate a copious secretion from the stomach (Bowie & Vineberg, 1935; Bucher *et al.* 1941), it has been commonly supposed that it is itself the secretagogue, but there is also some evidence that a histamine-free hormone, 'gastrin', may be the agent concerned (Komarov, 1938). A third phase, the 'intestinal', succeeds the gastric phase and, like it, is of a chemical nature; it is suggested that as the products of gastric digestion enter the duodenum, secretagogues are absorbed from them into the blood stream and pass to stimulate the gastric glands, while there is evidence also for the cooperation of secretin (Pratt, 1940). The term 'secretagogue', it may be added, is applied to 'any substance which excites secretion no matter by what mechanism the secretion is ultimately brought about' (Best & Taylor). The vagus may be regarded as the secretory nerve for the peptic, parietal and mucoid neck cells of the proper gastric glands, and also for the cardiac and pyloric glands (Bowie & Vineberg, 1935; Jennings & Florey, 1941), while the sympathetic nervous system has also been reported to influence the secretion of gastric mucus (Baxter, 1934).

Few investigators have as yet concerned themselves with the problem of the control of gastric secretion in the lower vertebrates, and such conclusions as emerge from their work are still very tentative. Dobreff (1927) found no evidence for a psychic phase in the gastric secretion in the dogfish (*Scyllium canicula*, etc.); furthermore, intramuscular injection of pilocarpine, acetylcholine and atropine did not affect the secretion of the gastric juice, and he concluded that the control of the secretion must be humoral, although histamine also had no effect.

Similar results were obtained by Babkin *et al.* (1935*a*), who, in a series of acute experiments on *Raja diaphanes* and *R. erinacea*, were unable to obtain any evidence for nervous control of the secretion. The only result of adrenaline injections was in some instances an inhibition of the normal secretion, perhaps as a result of vasoconstriction, while subcutaneous histamine injections had no effect. One interesting positive result, confirmed by MacIntosh (1935), was the obtaining of a continuous 'paralytic secretion' starting one or several days after complete destruction of the spinal cord. Merely cutting the cord below the medulla did not produce this effect, the cause of which is obscure, although the authors are inclined to interpret it as depending on the lowered tonus and increased permeability of the vessels supplying the mucosa. In contrast to these results, Ungar (1935), by perfusing the isolated stomach of *Squalus vulgaris*, *Scyllium canicula* and *Torpedo marmorata*, obtained evidence for some identity between the selachian and mammalian mechanisms; it was possible to collect 0.2 c.c. of a viscous, colourless juice per 10 min., and the volume of the presumed secretion could be increased by adding histamine or acetylcholine to the perfusing fluid, atropine inhibiting the effect of the latter drug.

Observations on the control of gastric secretion in Actinopterygii are virtually non-existent, but Bayliss (1935) identified pepsin in the gastric juice of *Pleuronectes* secreted under the influence of pilocarpine injections.

Smirnoff (1922), working on frogs with fistulae, found no evidence for a psychic phase, secretion requiring the actual presence of food in the stomach, beginning 40–50 min. after the entry of an insect, and depending upon the sympathetic nervous system. Langley (1881) had found that if a frog were fed a piece of sponge, the mechanical stimulation of this on the wall of the stomach produced a continuous discharge of secretion from the oesophageal glands, the regeneration of granules not starting until after at least some days, unless the sponge were removed, when regeneration started at once. Sponge did not have this effect on the gastric glands, where the presence of a worm was a more effective stimulus (suggesting perhaps a chemical effect), but it appeared to cause the secretion of acid. This mechanical effect was utilized by Smirnoff (1922); by placing cork or rubber in the stomach he obtained a secretion which was not inhibited by the section of the vagi. He claimed that the later stages of gastric secretion are determined in the frog chemically by the presence of proteolytic digestion products, and that the passage of food into the intestine is controlled by the acidity of the stomach contents, but as his work has been available only in abstract form the evidence on which these statements are based is unknown. According to Friedman (1934, 1937) both oesophageal and gastric secretion in *Rana esculenta* are primarily under the control of the sympathetic nervous system, the vagus, unlike the condition in mammals, playing no part.

Delrue (1930, 1933) developed a method for maintaining the gastric mucosa of the frog alive for as long as 29 hr. Starting with distilled water on the glandular side ('inside') and Ringer on the other ('outside'), the strip was able to set up a pH of 1.6–4.8 (usually 4.2–4.8) in the water, thus indicating a secretion of acid by the gastric glands. Finding that the normal gastric contents had a pH range of 1.9–4.9, he concluded that the excised mucosa was producing an effect very similar to that of its normal secretory activity. That it was a true secretion and not merely a diffusion of ions was shown by placing Ringer on both sides of the strip; after 2 hr., the pH on the 'outside' had risen from 7.3–7.4 to 7.5–7.6, while that on the 'inside' had fallen to 7.1–7.2. Gray *et al.* (1940) have confirmed this work and have incorporated some improvements into the method. They find an acid secretion sufficient to lower the pH of a saline solution to an average value of 2.5, with a minimum of 1.6; beginning some 2 hr. after the mounting of the mucosa, the secretion comes to an end after 6 hr. They provide additional evidence for regarding this as true secretory activity, for not only do they find, as did Delrue, that mucosa from a 'winter' frog forms very little acid under these conditions, but they also show that non-gastric membranes such as the oesophageal mucosa fail to produce any acid at all, while the 'outside' (non-secretory) surface of a gastric strip produces only alkali. Delrue showed that the addition of pilocarpine to the 'outside' fluid did not affect the secretion, but when combined with a stomach extract it caused a rapid increase of hydrogen ions, and then death of the tissue. The addition of histamine caused a slow increase of hydrogen ions over some hours, and other workers

have also obtained some evidence for its secretagogue action in the frog. Keeton *et al.* (1920), using bullfrogs with gastric fistulae, showed that the injection of either histamine or 'gastrin' extracts into the dorsal lymph sac stimulated the output of an acid gastric juice; in one experiment 0.2 mg. of histamine evoked production of 2.4 c.c. of juice in 2 hr., with from 0.20 to 0.27 % free hydrochloric acid. Friedman (1937) found that histamine stimulated acid and pepsin secretion in *Rana esculenta*, injections resulting in the production of a juice with high acid and low pepsin content from the stomach, and also in the secretion of pepsin from the oesophageal glands. Popielski (1929) had previously shown that the effect of histamine was increased by warming the frogs over several weeks to 37° C., *R. temporaria* being the most resistant to this treatment. The action of drugs will be further discussed in the next section, but so far as histamine is concerned it may be emphasized here that there is no reason for supposing that such a stimulant of mammalian secretion will necessarily produce a similar effect in another group of vertebrates, nor, if it is actually found to do so, does it follow that this is a normal physiological process in that group. A significant illustration of this is the fact that histamine has no effect on the blood pressure of the skate (MacKay, 1931), and does not have a dilator effect on frog capillaries; it has been possible, however, to extract from frog's skin a substance which will dilate the capillaries and which, while not actually histamine, does resemble it chemically and physiologically (see Best & McHenry, 1931). It seems likely enough that similar conditions may be found to exist in the digestive system of the lower vertebrates. It must be remembered, too, that even when a mammalian drug is active in a poikilotherm, the lower metabolic rate may greatly modify the animal's response; thus the effect of insulin may be greatly delayed and prolonged in such forms in contrast with its reaction time in mammals, and MacKay found that while adrenaline, choline, acetylcholine and pituitrin had pressor effects on the blood pressure of skates, these were more prolonged than in mammals. All these considerations may help to account for the contradictory results obtained by different workers on the lower vertebrates.

VI. THE CONTROL OF GASTRIC MOVEMENT

Accounts of the anatomy of the nerve supply to the stomach in fish have recently been given by Young (1931, 1933) and by Babkin *et al.* (1935*b*). Müller & Liljestrand (1918), working mainly on *Raja clavata*, found that the spontaneous gastric movements in operated animals began near the pylorus and advanced anastaltically over the pyloric region and sometimes on to the corpus, showing much variation in frequency and rate of progression. Occasional catastatic movements occurred, beginning at the middle of the stomach, while mechanical stimulation set up strong movements, especially near the pylorus; movements were much stronger when the brain and spinal cord were destroyed. In *Mustelus* the corpus and pyloric regions are described as looking and acting like distinct organs (Alvarez, 1927), contractions on the former very rarely passing on to the latter, the musculature at the point of interruption between the two being broken by connective tissue, as

it is at the pylorus; deep forwarding contractions are never seen. In the ray, however, there is less distinction between the parts of the stomach, and deep peristaltic waves are occasionally seen. In *Scyllium canicula*, spontaneous catastatic movements are seen on the corpus (Young, 1933).

Stimulation both of the vagus and of the anterior splanchnic nerve in *Raja diaphanes*, *R. stabuliformis*, *R. erinacea* and *Squalus acanthias* produces a motor reaction, with the sympathetic producing the dominant effect. Both catastatic and anastaltic waves are seen, the latter beginning near the pylorus after a variable latent period and extending over the whole stomach. Vagal stimulation produces less movement, and this does not necessarily arise near the pylorus but occasionally even in the upper part of the corpus; sometimes the vagus has a definite inhibitor effect (Müller & Liljestrand, 1918; Lutz, 1931; Babkin *et al.* 1935*b*). As would be expected from these results, adrenaline has in general a stimulating effect on stomach strips of rays and dogfish, causing a rise in tonus and sometimes increased motility of all parts of the stomach of *Raja* and *Squalus* (Dreyer, 1928; Lutz, 1931; Nicholls, 1933).

Conditions in the teleosts are a little variable, but here again both autonomic systems appear to have motor effects. In *Anguilla* (Müller & Liljestrand, 1918), vagal and sympathetic stimulation both produce catastatic waves beginning some centimetres from the pylorus, the sympathetic effect being the stronger. In *Esox* the vagus increases the tone and causes longitudinal contraction of the stomach, while the sympathetic induces catastatic waves in the lower part of that organ; the vagal contraction is here the more marked, but only occasionally produces small catastatic waves. In *Perca* the vagus produces strong, the sympathetic weaker, movements. In *Uranoscopus* and *Lophius* the vagus causes marked contraction of the well-developed longitudinal musculature of the stomach; the splanchnic nerve has a slight and rather doubtful motor effect (Young, 1936).

As regards the frog, there seems to be good evidence for differences between the musculature of the corpus and pyloric regions in respect of such properties as excitability and frequency of contraction (Alvarez, 1917; Gellhorn & Budde, 1923; Budde & Gellhorn, 1924). The pyloric region is the more active in the isolated stomach of freshly killed animals (Babkin, 1924) and its movements, observed in the filled stomach in situ, have been considered suitable for kneading the food and driving it into the intestine (Grützner, 1905). The effect upon movement of variations in the acidity of the contents would, of course, be of importance under normal conditions, and Babkin showed that while 0.05% hydrochloric acid increased the contractions and reduced their frequency, 0.1–0.2% weakened or arrested them, causing a marked lowering of tone, the pyloric region proving to be the more sensitive to the acid. Sodium carbonate raised the tone, increased the frequency and intensity of the contractions, and stimulated the activity of the pyloric region. It is difficult to judge how far such differences in properties are actually manifested under physiological conditions, but some observations of Patterson (1916, 1928, 1933) suggest that the normal gastric reactions may well be simpler and more constant in form than they are under experimental conditions.

Using a balloon placed in the stomach and connected through a pharyngeal opening with a water manometer, he found that fasting *Rana catesbiana*, and also *Necturus*, showed hunger contractions which proceeded with definite regularity for day after day, although they could be inhibited by 1 % sodium carbonate and 0.5 % hydrochloric acid, or, in the frog, by appropriate distension of the lungs (Scantlebury & Patterson, 1939); in the latter animal each occupied 1.6 min., with 16–33 sec. intervals. By the use of a bismuth balloon and X-rays, these contractions were found to be peristaltic, originating within about 1 cm. of the cardiac end and advancing over the entire stomach, increasing in strength as they proceeded; Dixon (1902) had earlier claimed that they were rings which did not travel. In man the hunger contractions differ in certain respects from the normal digestive peristalses (there are, for example, tonus changes which are not found in the frog), and it is indicative of the greater simplicity in *Rana* that feeding caused little if any change in the movements in that animal. The inhibition induced by alkali and acid was effected more slowly than in man and the dog, suggesting that the gastric reflex was less efficient; moreover, these substances were not effective when placed in the mouth, although the sight of a small frog caused inhibition.

There is much disagreement in the literature as to the way in which tonus regulation is achieved in the frog. Dixon (1902) stated that faradic stimulation of the sympathetic increased the tonus of the intact stomach of *Rana temporaria* and augmented the automatic contractions, while vagal stimulation lowered tonus but had no clear effect on movement; it thus appeared that tonus might normally be regulated by a balanced action between the two autonomic components, although of a reversed type from that found in mammals. Itagaki (1927, 1930), however, while confirming the sympathetic effect in *R. esculenta*, found that the vagus also increased tone, and claimed that inhibitor fibres in the sympathetic system completed the tone-regulating mechanism, a conclusion which received some support from Gruber's (1923) observation that adrenaline in weak concentration caused contraction of the stomach, but in strong concentration caused relaxation. Hopf (1910), however, had previously shown that vagal stimulation in the frog had at first an inhibitory and then a motor effect, the latter being the stronger. If the stomach were filled with acid, the motor effect preponderated, but if with alkali then the stomach relaxed, the effect of vagal stimulation thus depending upon the precise condition of the stomach. So far as the effect of drugs is concerned, Epstein, using *Xenopus* (1931, 1932*a*) and later *Rana* and *Bufo* (1932*b*), found that the responses of strips to pilocarpine, atropine, arecoline and adrenaline were very similar to those of mammalian tissues, and concluded that the parasympathetic system was mainly motor to the oesophagus and stomach. Yüh (1931), following up the work of Itagaki, found evidence for excitatory and inhibitory action of the sympathetic system in *Rana esculenta*, for adrenaline, applied either to strips or to the serosa of the whole organ, reduced the tone and the amplitude of contraction, while faradic stimulation of the sympathetic was usually excitatory but sometimes inhibitory. Patterson (1928) had concluded from experiments on the intact stomach of *Necturus* that the vagus was predominantly inhibitory and the sympathetic possibly excitatory,

but Friedman (1935), applying drugs to strips from the same animal, obtained results which were very variable.

It is clear that these findings contain contradictions which it is impossible to resolve on the available evidence. However, the influence of the autonomic system on gastric movement in the lower vertebrates is well established, and it seems possible to conclude that both sympathetic and parasympathetic systems often act in the same sense, with the first having perhaps the more effect. At first sight this situation might not seem very different from the condition in mammals, where the reciprocity between the two is not absolutely invariable (McSwiney, 1931), but in fact the evidence suggests that there does exist, at least in fish, a fundamental difference, for Young (1936) finds in that group that it is impossible by pharmacological, physiological or morphological criteria to differentiate between the two systems. He has suggested that this could be explained if it be assumed that each segment of the vertebrate body had originally an outflow of visceral fibres conveying motor impulses to the alimentary canal and corresponding to the present sympathetic system, the vagus later extending backwards so that there arose an overlapping, and later a reciprocity, between two systems corresponding to the present two autonomic divisions. Support for this view is found in the fact that in *Scyllium* (Young, 1933) only the corpus responds to vagal stimulation, whereas in man the vagal influence extends over the whole of the stomach and small intestine and probably on to the colon (Alvarez, 1940). According to Brandt (1922), however, the vagus extends to the hind end of the intestine in *Myxine*.

How far similar considerations may account for the contradictions obtained in work on the Amphibia is not yet clear; Campenhout (1930*a*, 1930*b*) has concluded on embryological grounds that in *Rana pipiens* and *R. palustris* the alimentary canal obtains only a sympathetic innervation, the visceral branch of the vagus being entirely sympathetic in origin, but it must be remembered that at least the cardiac branch of that nerve is well known to be cholinergic. However, the above discussion should serve to show that experimental work on the gastric innervation in the lower forms demands cautious interpretation, and the results, both physiological and pharmacological, may well be expected to conflict with those obtained for mammals. This will naturally apply with equal force to analysis of the nervous control of secretion, discussed in the previous section.

VII. ABSORPTION

Indications of the existence of gastric enzymes additional to pepsin have already been noted, and there is some complementary evidence for the occurrence of absorption in the stomach. Herwerden (1908) found fat in the gastric epithelium and in the submucosa and lymph vessels of Selachii and teleosts which had been killed during digestion, but not in fasting animals, and considered this as evidence for the absorption of fat; Haus (1897) had previously suggested the same possibility as a result of observing fat droplets in the gastric epithelial cells of *Anarrhichas*, while Blake (1930) has recently noted similar indications in *Centropristes*. Fat

droplets become visible in the gastric epithelium and neck cells in *Oncorhynchus* (Greene, 1912*b*) after rectal feeding with olive oil, although they are much more abundant in the intestine and pyloric caeca; in *Pleuronectes* also there has been described a marked increase in the fat content of the gastric epithelium after feeding (Dawes, 1930). Van Slyke & White (1911) fed dogfish on chopped beef, and claimed that after 6 hr. at least 20% of the ingested nitrogen had been absorbed from the stomach; Soulima (1919), however, found no evidence for nitrogen absorption from the stomach of *Scyllium catulus*. Kingsbury (1894) showed that the gastric epithelial cells of *Necturus* contained fat droplets when the stomach contained food, but not when the whole alimentary canal was empty.

These facts make it probable that some absorption, at least of fats, occurs in the stomach, but at present too little is known of the factors which would govern such a process. Conditions in the lower vertebrates clearly provide for the slow dissolution of the food, but it is not known whether the gastric movements are such as to drive the early products of digestion into the intestine as they are formed, or whether they remain for some time in the stomach. It is, however, suggestive that in *Pleuronectes* (Dawes, 1930) there is probably a transference of absorption from the stomach into the intestine when the frequency of meals is increased, a condition which has been shown to result in a more rapid passage of food through the stomach. The possession by many fish of a well-demarcated pyloric region, with the characteristics outlined earlier, would also seem to facilitate the possibility of absorption, and it is of interest that in *Oncorhynchus* (Greene, 1912*b*, 1913) more fat droplets are found in the cells of the pyloric region, where the acidity may be expected to be lower, than in those of the corpus.

It may be noted that the possibility of the final stages of digestion being intracellular cannot be excluded. Van Slyke & White (1911) found that digestion of protein in the dogfish did not proceed, either in stomach or intestine, beyond a stage midway between tri- and di-peptides, and Vonk (1927) has suggested that an intracellular erepsin might complete the process. There are, in fact, several indications that dipeptidases may be intracellular in their action (Linderstrøm-Lang, 1939), and, as has been mentioned earlier, Wright *et al.* (1940) have shown that some of the mammalian intestinal enzymes are present in the succus entericus in intracellular form.

VIII. STOMACHLESS FISH

A stomach is lacking, as far as histological structure is concerned, in the Dipnoi (Yung, 1899; Pernkopf & Lehner, 1937), the Holocephali (Fahrenholz, 1915), and in certain members of the Teleostei (Table 1). Thus, in *Cyprinus carpio*, which typifies this condition, the oesophagus is a short, muscular tube with ten to twelve longitudinal folds continuous with those of the intestine and lined by a stratified epithelium, with goblet cells, which passes into the columnar intestinal epithelium (Curry, 1939). Through most of its length the intestine has a diameter of 3 mm., but at its anterior end it widens to 10 mm., and thus forms a food receptacle which has often been mistaken for a stomach; no gastric glands are present, however, and

the bile and pancreatic ducts enter within 6 mm. from the oesophagus. There is apparently no valve present between the latter and the intestine. Complete absence of peptic digestion has been demonstrated in this animal (Kenyon, 1925; Beauvalet, 1933 a), in *Tinca tinca* (Beauvalet), in *Calotomus*, *Thalassoma*, *Spheroides* and *Salarias* (Ishida, 1936), and in *Fundulus heteroclitus* (Babkin & Bowie, 1928). *Mugil cephalus* is included here as a physiologically stomachless form; its stomach is described by Ishida (1935) as muscular, like the gizzard of a fowl, with no gastric glands or hydrochloric acid, but with trypsinogen, amylase, glycogenase, maltase and invertase.

Table 1. *Well authenticated examples of the absence of a stomach in the Teleostei*

References: (1) Oppel (1896); (2) Jakobshagen (1911); (3) Follmann (1927); (4) Schacht (1931); (5) Babkin & Bowie (1928); (6) Ishida (1936); (7) Beauvalet (1933 a); (8) Curry (1939); (9) McVay & Kaan (1940); (10) Pictet (1909); (11) Pietruski (1914); (12) Rogick (1931); (13) Kenyon (1925); (14) Yung (1899); (15) Pilliet (1885).

Acanthopsidae.	<i>Cobitis fossilis</i> (1, 14).
Blennidae.	<i>Blennius pholis</i> (1, 15); <i>B. tentacularis</i> (11); <i>Salarias enosima</i> (6).
Callionymidae (2).	Genera not named.
Cyprinidae.	<i>Cyprinus carpio</i> (1, 3, 7, 8, 13); <i>Tinca vulgaris</i> (1, 10); <i>T. tinca</i> (7); <i>Leuciscus dobrylus</i> (1); <i>L. rutilus</i> (10); <i>Phoxinus laevis</i> (1, 14); <i>Squalius cephalus</i> (3); <i>Chondrostoma nasus</i> (3); <i>Barbus fluviatilis</i> (3, 10); <i>Carassius auratus</i> (9, 10); <i>Camptostoma anomalum</i> (12).
Cyprinodonti.	<i>Fundulus heteroclitus</i> (5); <i>Gambusia affinis</i> and all other forms examined (4).
Gobiesocidae.	<i>Lepadogaster bimaculatus</i> (1, 2, 15).
Gobiidae.	<i>Callionymus lyra</i> (1, 15).
Labridae.	<i>Labrus bergyllia</i> (1, 15); <i>Crenilabrus pavo</i> (1, 15); <i>Calotomus japonicus</i> (6); <i>Thalassoma umbrostigma</i> (6).
Mugilidae.	<i>Mugil cephalus</i> (6; see text).
Scaridae (2).	Genera not named.

It would be of interest to know how far the absence of a stomach affects the efficiency of utilization of ingested material. The stomachless roach is said to retain a slightly lower percentage of the available nitrogen than the trout (Mann, 1935). Owing to the absence of a stomach for storage, it can take in at one meal only 4-5 % of its total weight, whereas the trout can take 20 %, but this advantage is partly counteracted by the fact that the presence of such a bulk of food in the stomach slows down proteolytic digestion, since the pepsin can only attack the surface (see p. 10), and thus while the roach after 28 hr. has only 30 % of the original nitrogen still present in insoluble form, this condition is not reached until 52 hr. in the trout, in which animal the nitrogen does not begin to become soluble to any marked degree until after 20 hr. (at 12-15° C.). In the stomachless *Gambusia affinis* (Sokolov & Chvaliova, 1936) a meal of *Anopheles* larvae is completely discharged from the anterior receptacle (incorrectly regarded as a 'stomach' by the authors) in 3-6 hr., while the time of passage of food through the gut in one-year-old carp ranges from 18 hr. at 10° C. to 4½ hr. at 26° C. (Maltzan, 1935). It is clear, then, that food does not remain for so long in the anterior receptacle as it has been seen to do in a true stomach, and the facts indicate that the absence of the latter would involve the taking of smaller and more frequent meals. How far the absence of acid limits the

utilization of prey with calcareous exoskeletons is not known, but effective trituration seems to be possible, for Herwerden (1908) records that mollusc shells, which often fill the fore-gut in the stomachless *Blennius*, are found in a finely ground condition in the hind-gut.

The absence of a stomach in certain teleosts must be a secondary condition, since that organ is widely developed not only in the group itself, but in the lower Actinopterygii, and it would be of interest to investigate further the question of the existence of transitional stages in its loss. One suggestive example is *Zoarces anguillaris* (MacKay, 1929); this has an anatomical stomach with pyloric sphincter, but the internal pH ranges from 6.5 to 8.4, and mucosa extracts contain, in addition to a presumed pepsin, which it is supposed could hardly be operative in such a medium, a strong lipase and a very weak amylase. Again, the stomach of *Gobius niger* is said to have groups of reduced gastric glands in the form of small acini, each with six to eight granular cells (Edinger, 1877). Such cases require further investigation, but it may well be that the tendency to low acidity in the teleostean stomach, which has already been noted, may have led in more than one family to an increasing dependence on intestinal digestion and thus to the loss of the stomach. This loss cannot, on present information, be correlated with feeding conditions (*Calotomus* feeds on algae and *Spheroides* on Crustacea and molluscs, for example; Ishida, 1936), but it would be of interest to know how readily a fish without a stomach could utilize large prey.

As for the Holocephali, their apparent close relationship with the Selachii (Watson, 1937) makes it almost certain that here too the absence of a stomach is secondary (although Pernkopf & Lehner (1937) seem to regard it as primitive), and the Dipnoi are best interpreted in the same way, for a contrary view involves the difficult assumption that the stomach has evolved within the Pisces, and probably independently in more than one line. It should be remembered that both groups are highly specialized, and Dean (1906) has, in fact, interpreted the absence of a stomach in the Holocephali as a secondary result of an early shortening of the visceral cavity.

IX. SUMMARY

True gastric digestion, involving the action of pepsin, appears to be found in animals only in the Chordata, and in this group, at the present day, only from the Pisces upwards. It is suggested that its appearance may be correlated with the change from microphagy to macrophagy which occurred in the early history of the phylum.

Information relating to gastric digestion in the lower vertebrates is still too limited for any extensive generalizations to be possible, but many differences between conditions in these forms and in the mammals are evident. Certain facts illustrating the more primitive organization of the gastric mucosa in fish (and to a lesser extent in Amphibia) are briefly summarized.

There seems to be a general resemblance between the pepsin of mammals and that of the lower vertebrates, with perhaps some minor adaptation to medium.

There is some positive evidence for the existence of enzymes additional to pepsin in the stomach of fish, but the source of these has not been made clear.

An acid fluid has been obtained from the stomach of fish and Amphibia by siphon or fistula. The secretion of acid may be continuous throughout life in selachians, but increases after feeding; in rays an alternation of acidity and alkalinity has been reported. The stomach of teleosts seems to be less acid than that of selachians and Amphibia, but the pH at the surface of ingested prey may be more suitable for the action of pepsin than is that of the general contents. Digestion proceeds much more slowly in poikilotherms than in mammals, and the food commonly remains in the stomach for one or more days.

The few observations at present available suggest that gastric secretion in selachians, unlike that of mammals, may be controlled entirely by humoral means, but the evidence is incomplete and not altogether consistent. There is no evidence for a 'psychic phase' in selachians or Amphibia, but there are indications that in the latter group the sympathetic nervous system may be an important factor in the control of secretion. Histamine is reported to stimulate the secretion of acid and pepsinogen in frogs.

Extensive studies of the nervous control of gastric movements in fish and Amphibia have led to results which are often conflicting and difficult to reconcile with those established for mammals. Some possible explanations for this are discussed, and in particular it is emphasized that a clear-cut reciprocity between sympathetic and parasympathetic systems may be lacking in the lower vertebrates.

There is evidence for the absorption of fat in the stomach of fish, and it is pointed out that certain characteristics of gastric digestion in poikilotherms would appear to make that organ more adapted for absorption than it is in mammals.

It is concluded that the absence of a stomach from the Holocephali, the Dipnoi and certain Teleostei is likely to be a secondary rather than a primitive condition.

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THE FUNCTION OF PHOSPHATE IN CELLULAR ASSIMILATIONS

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I. THE COUPLING OF PHOSPHORYLATION WITH FERMENTATION AND RESPIRATION

THIS report deals mainly with two fundamental publications from Warburg's laboratory (Warburg & Christian, 1939; Negelein & Brömel, 1939) and with some papers of equal importance by Lipmann (1939, 1940). If examined as isolated contributions these papers may appear to be very specialized; they deal with the formation of two new phosphorylated intermediates in fermentation and oxidation. However, if we examine these findings in the light of the studies of phosphorylations in the last 30 years, supplemented by some very simple thermodynamical considerations, we shall soon realize that the discoveries of the two new esters are not just experimental curiosities but actually represent the first decisive steps towards a solution of one of the most general problems in biology, namely, the nature of coupling between oxidative metabolism and cellular assimilations or syntheses.

It seems advisable before proceeding further to try to give a definition of the concept assimilation (or synthesis). This is not so easy, however, since a purely thermodynamical definition would hardly be able to describe completely the concept of assimilation. Nevertheless, I prefer to define assimilation on a thermodynamical basis. Assimilatory processes are biological reactions which are unable to occur spontaneously because they are endergonic.² Assimilatory processes can therefore proceed only when coupled with exergonic processes which are also known as dissimilatory. We have numerous examples of such a coupling between assimilatory and dissimilatory reactions; in fact almost all biological processes include both a dissimilatory and an assimilatory component. The formation of polyhexoses from monohexoses and of sugars from lactic acid and the esterification

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² C. D. Coryell (1940) recently suggested that the old terms exothermic ($-\Delta H$) and endothermic ($+\Delta H$) should be used only to state changes in heat content, whereas changes in free energy should be designated by the terms exergonic ($-\Delta F$) and endergonic ($+\Delta F$).

of numerous organic compounds with phosphate are well-known assimilatory processes. As an example of an endergonic process which we would hardly term assimilation, we have the relaxation of the contractile structure in muscle. Processes of the latter kind are usually described as 'charging'.

This article will deal mainly with one important kind of assimilatory process, the phosphorylations, and their coupling with dissimilatory processes like fermentations and respiration.

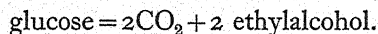
It is a well-known fact that phosphate occurs in large amounts inside cells and is the most important intracellular anion. Most of the intracellular phosphate is in organic combination. We are in this connexion mainly concerned with the water-soluble phosphoric esters, especially phosphocreatine, the adenine-nucleotides and the sugar phosphates. The first observation that phosphate is connected in some way or other with metabolism goes as far back as 1905 when Harden & Young discovered the uptake of phosphate in cell-free fermentation (juice from dried yeast).

Harden & Young (1906) found the following differences between cellular (intact yeast cells) and cell-free (yeast juice) fermentation:

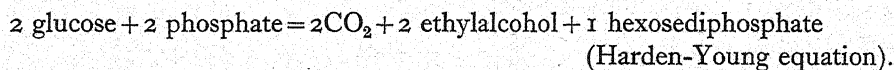
(1) Yeast cells do not utilize added inorganic phosphate to any appreciable extent. Yeast juice utilizes phosphate; for every mol. CO_2 and alcohol formed 1 mol. inorganic phosphate disappears.

(2) Addition of phosphate is unnecessary in fermentation carried out by yeast cells. Addition of phosphate is necessary for fermentation carried out by yeast juice; when the inorganic phosphate is used up the 'cell-free fermentation' stops before all the glucose is consumed. A further addition of phosphate starts the 'cell-free fermentation' again.

(3) The 'cellular fermentation' converts glucose according to Gay-Lussac's equation:



In the 'cell-free fermentation' only half of the glucose which disappears is converted into CO_2 and alcohol; the other half appears as hexosediphosphate:



(4) Addition of small amounts of arsenate converts the 'cell-free fermentation' from the 'Harden-Young type' to the 'Gay-Lussac type' (Harden & Young, 1911).

The ratio between esterified P and CO_2 formed is surprisingly constant provided the yeast juice is always prepared in the same manner. Small variations in the preparation give rise to less P esterification owing to formation of hexosemono-phosphate (Robison-ester) (Robison, 1922; Kluyver & Struyk, 1928; Smythe, 1937) instead of hexosediphosphate. Nilsson & Alm (1936) have been able to prepare dried yeast which ferments glucose like living yeast cells, i.e. according to the Gay-Lussac equation. Further addition of large amounts of phosphate gradually converts the fermentation by the Nilsson yeast from a typical 'Gay-Lussac fermentation' into a more or less typical 'juice fermentation' ('Harden-Young type'). This puzzling phosphate effect is probably due to an inhibition of a phosphatase (adenylpyrophosphatase?).

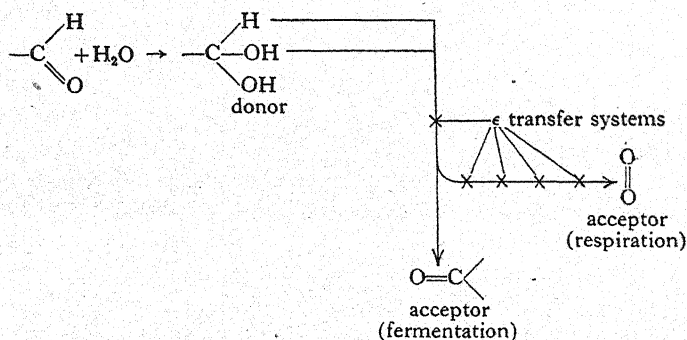
The interesting conversion of 'Gay-Lussac fermentation' into 'Harden-Young fermentation' by phosphate and the conversion of the latter type back to the first by arsenate show clearly that although the large accumulation of hexosediphosphate which we find in the 'cell-free fermentation' is not seen with intact cells, it is just a simple variation of the cellular fermentation. Perhaps the accumulation is attributable to lack or inhibition of a single enzyme system, which in the living cell diverts the phosphate to other substances than glucose. Thus, the large accumulation of hexosediphosphate which we find in cell-free systems does just represent a one-sided utilization of the phosphate taken up in the fermentation.

Glycolytically active muscle extracts are also able to phosphorylate sugars, but only polyhexoses; the sugar phosphoric esters accumulate in intact muscles as well as in muscle extracts when specific enzyme poisons, like iodoacetate or fluoride (Lundsgaard, 1930; Embden & Deuticke, 1934), are added. In intact muscles phosphorylations on a large scale, in particular of creatine, can be observed in the recovery period after a contraction. Lundsgaard (1930 *a, b*) discovered that the rephosphorylation of creatine is coupled to fermentation (glycolysis) or respiration. He found the relationship between creatine phosphorylation and lactate formation to be very constant: 2 mol. phosphate are esterified to phosphocreatine per 1 mol. lactate formed from glycogen (Lundsgaard equation).¹

What is the chemical explanation of such a coupling between fermentation and phosphorylation?

The coupling between oxygen consumption and phosphorylation observed by Lundsgaard (1930 *a, b*), Engelhardt (1930), Runnström *et al.* (1934) and several other investigators indicates that the stoichiometrical coupling between fermentation and phosphorylation is connected with the oxido-reductive phase of fermentations.

Pasteur, in 1861, had already anticipated that fermentations are oxygen-free oxidations. Kluyver & Donker (1926) combined the hypothesis of Neuberg and of Wieland and advanced the idea that all fermentations involve transfer of hydrogen from a donor, the sugar, to an acceptor formed in the fermentation process (Neuberg). According to Wieland, carbonyl groups are oxidized as hydrates:



¹ Lipmann (1941) calls attention to the fact that the ratio $\frac{2 \text{ mol. phosphocreatine formed}}{1 \text{ mol. lactate formed from glycogen}}$ could be reached if hexosemonophosphate is phosphorylated to hexosediphosphate by another molecule of hexosemonophosphate, thus sparing the phosphocreatine.

Wieland's aldehydehydrate hypothesis has been of much importance for the classical scheme of biological oxidations as dehydrogenations, but has, as we shall later understand, not been able to throw any light on the nature of the coupling between biological oxidations and syntheses.

When studying the nature of respiration and fermentation we have to deal with three main problems, the formation of the electron donor (reductant), the formation of the electron acceptor (oxydant) and the mechanism of transfer of electrons from donor to acceptor.

The *acceptor* problem was first solved.¹ In respiration the acceptor, oxygen, is taken up from the environment. In fermentation the nature of the acceptor was clarified by the classical experiments of Neuberg & collaborators who showed that the acceptors in fermentations are carbonyl groups. In fermentations the acceptor has to be formed from the original substrate. Generally speaking, in fermentations the acceptor is formed and regenerated from the substrate by a kind of anhydride reaction by which water is removed from the first or the second oxidation product (oxidation level) of the original substrate.

The mechanism of electron *transfer* has been completely clarified by the brilliant experiments of Warburg & collaborators (1930-8), who isolated and identified the nature of the electron transfer systems (see Warburg, 1938; Warburg & Christian, 1938 *a, b*). In fermentations electrons are transferred by pyridine-nucleotides.

The *donor* problem is the most complex of the three; it remained mysterious until the year 1939, when the investigations of Warburg & Christian and of Lipmann showed that carbonylphosphate represents one of the most important electron donors in biological oxidations.

The most important carbonyl oxidations, sugar oxidation and pyruvate oxidation, are connected in some way or other with the uptake of inorganic orthophosphate. When we examine the mechanism of sugar oxidation where phosphate plays such an outstanding role we have to distinguish between two types of phosphate effect. The first type is the effect of an ordinary ester linkage on the rate of enzymic sugar oxidation. Most biological systems do not oxidize glucose or triose but 6-phospho-glucose and 3-phospho-triose. The role of the phosphate esterified in the C₆ or C₃ positions is not completely known but is probably concerned with the binding of the sugar to the specific catalytically active protein. Warburg & Christian, who have isolated the pure triose oxidase, observed that 1% of the pure enzyme gives the same rate of oxidation when 3-phosphoglyceraldehyde is substrate as 100% protein when glyceraldehyde is substrate. The protein seems to decrease the activation energy (1938).

The phosphate in the C₃ position will furthermore become of essential thermodynamical importance when the polyalcohol structure of the sugar acid 'degenerates' to the α -keto structure of pyruvic acid.

The large fall in free energy of this process (cf. Lipmann, 1941) will be discussed at another place in this review.

¹ This might be attributed to the fact that a double bond probably represents an activated state, exhibiting paramagnetic properties (cf. Lewis, 1923).

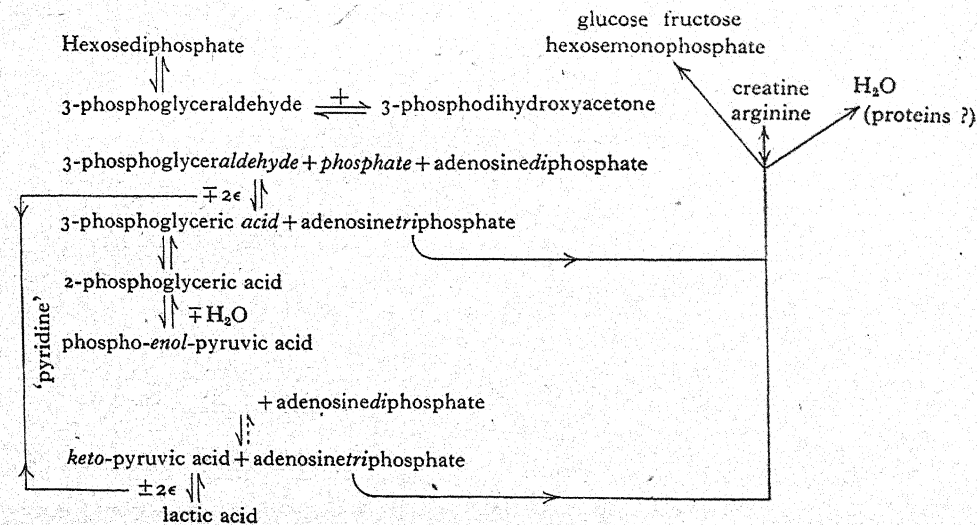
The second type of phosphate effect which has been found at least in two important biological oxidations, the phosphotriose and pyruvate oxidations, is the most important in the problem of biological syntheses: triosephosphate and pyruvate oxidation cannot occur unless inorganic phosphate is present (Meyerhof *et al.* 1937; Lipmann, 1937).

In the triosephosphate oxidation we have a compulsory coupling between oxidation and phosphorylation, i.e. oxidation can only proceed if inorganic phosphate is taken up, and this uptake of phosphate depends on oxidation.

Warburg (1938), and other investigators after him, found that the oxidation step for which phosphate is necessary is the transfer of electrons from phosphoglyceraldehyde to the pyridine-nucleotide; the phosphate is taken up and is found bound in the pyrophosphate of the adenine-nucleotide, which has to be present in this oxidation process as a phosphate acceptor.

The equation is the following (Meyerhof *et al.* 1937; Needham & Pillai, 1937):
 phosphoglyceraldehyde + phosphate + adenosinediphosphate + pyridine-nucleotide
 \rightleftharpoons phosphoglyceric acid + adenosinetriphosphate + reduced pyridine-nucleotide.

Thus knowledge of the phosphate phenomenon observed by Harden & Young in 1905 was amplified to a considerable extent though it was not at all understood. The position of the compulsory coupling between oxidation-reduction and phosphorylation in the Embden-Meyerhof fermentation scheme can be illustrated as follows:



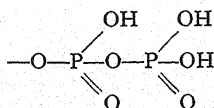
II. SYNTHESIS OF SUGAR FROM LACTATE

The reverse of glycolysis, i.e. formation of sugar from lactate, has been demonstrated in cell-free extracts of muscle and yeast (Green *et al.* 1937; Meyerhof *et al.* 1938b). Starting with lactate as electron donor (reductants) and phosphoglyceric acid as acceptor, Green *et al.* were able to get phosphotriose, provided this was trapped by cyanide or semicarbazide; inorganic phosphate was liberated from an

unknown source. Meyerhof *et al.* identified the unknown phosphate donor as adenosinetriphosphate and thus demonstrated the complete reversibility of the oxidation-reduction illustrated in the scheme.

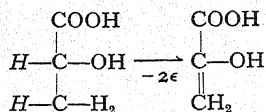
Phosphorylation and dephosphorylation of adenosinepolyphosphates are thermodynamically very important reactions. Unfortunately, the free-energy change for these reactions is not known. It is, however, of great significance that the heat change (ΔH) has been measured (Meyerhof, 1930). The ΔH of the dephosphorylation of adenosinetriphosphate, which is a splitting of pyrophosphate, amounts to 11,000 cal. per mol. phosphate. Latimer (1938) estimates the ΔF of pyrophosphate splitting as 'several tenths of a volt', i.e. more than 10,000 cal. per mol. P.¹ Since phosphocreatine is in an easily reversed equilibrium with adenylypyrophosphate (2-creatine + adenosinetriphosphate \rightleftharpoons 2-phosphocreatine + adenosinemonophosphate), the dephosphorylation of phosphocreatine amounts to about 10,000 cal. per mol. P. Thus, formation and hydrolysis of pyrophosphate which occur when adenine-nucleotides are phosphorylated or dephosphorylated are two of the most energy-rich step reactions in biological systems.

The energy of phosphotriose oxidation is used for formation of a new pyrophosphate group



The fact that the dephosphorylation of phosphopyruvic acid also is able to phosphorylate the adenine-nucleotide shows that the conversion of phospho-enolpyruvic acid into ketopyruvic acid is a very strongly exergonic process. Since these two reactions are strongly exergonic, the reverse reactions must be strongly endergonic, i.e. require energy in order to proceed; this energy is at least in the case of the reduction of phosphoglyceric acid delivered by the pyrophosphate of adenosinetriphosphate (Meyerhof *et al.* 1938b).

The synthesis of phospho-enolpyruvic acid from pyruvic acid has not yet been demonstrated. In kidney extracts, which under aerobic conditions show a very rapid phosphorylation of different compounds (Kalckar, 1937), pyruvic acid is not phosphorylated. It has, however, been possible to get phosphopyruvic acid when malate or fumarate is oxidized by kidney extracts. Both lactate and malate can be oxidized in such a manner that the enol compound which perhaps is the phosphate acceptor proper is formed primarily:



Although the formation of phosphopyruvic acid remains still rather obscure, we are able to get at least a slight idea of the possible mechanism of this reaction.

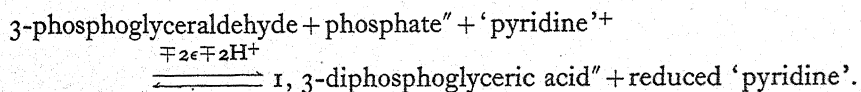
¹ Since inorganic pyrophosphate is an anhydride between two OH groups of the pK_s type, the change in free energy by splitting inorganic pyrophosphate is the same at pH 7 and at pH 0.

The mechanism of the coupling between the reduction of phosphoglyceric acid and dephosphorylation of adenylypyrophosphate (or the reverse reaction) remained, however, a complete mystery until Warburg and collaborators solved the problem in 1939. What has hitherto been described as energetic coupling, that oxidation of phosphotriose 'provides energy' for a phosphorylation of the adenine-nucleotide or vice versa, can now be described in precise chemical equations of thermodynamically perfect harmony.

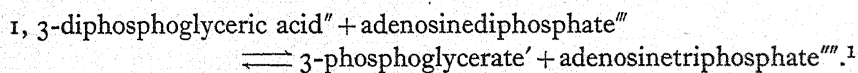
III. THE NATURE OF ENERGETIC COUPLING

Warburg & Christian (1939) were able to purify and isolate the enzyme which in the presence of phosphate catalyses the oxidation of phosphotriose to phosphoglyceric acid by the pyridine-nucleotide. This isolation bore great fruit because it enabled Negelein & Brömel (1939) to detect the primary oxidation product of phosphotriose. This product is 1, 3-diphosphoglyceric acid. The phosphate taken up in the oxidation process reappears in the carboxylic group.

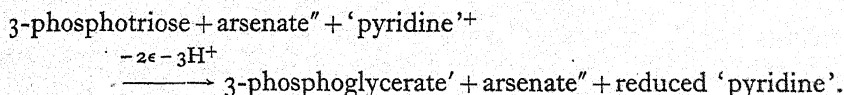
The equation for the oxidation process is therefore as follows:



The reaction was found to be very easily reversed. Addition of adenosine-diphosphate and another special enzyme causes the following reversible reaction:



Arsenate is able to replace phosphate and is active in very small concentrations since arsenate does not disappear in the oxidation process, presumably because the arsenylated carboxylic group splits spontaneously. The equation is therefore the following:



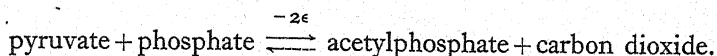
Negelein & Brömel's isolation of 1, 3-diphosphoglyceric acid gave rise to a new important discovery.

Lipmann (1939, 1940), working with a purified enzyme (from bacteria), found:

(1) Phosphate or arsenate is necessary for the pyruvate oxidation.

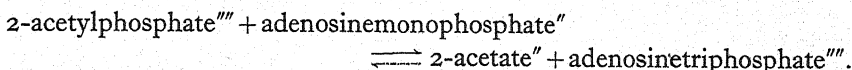
¹ If the redoxpotential of the system phosphotriose + phosphate \rightleftharpoons diphosphoglyceric acid can be obtained, the energy of the pyrophosphate linkage can very easily be calculated. The difference between the redoxpotential of this redox system together with the system diphosphoglyceric acid + adenosinediphosphate \rightleftharpoons monophosphoglycerate + adenosinetriphosphate and the redoxpotential of the same systems + the enzyme which catalyses the dephosphorylation of adenosinetriphosphate to adenosinediphosphate (an enzyme which is easy to obtain from lobster muscle) will yield the free energy of the pyrophosphate linkage.

(2) Acetylphosphate is the primary oxidation product, and the oxidation therefore proceeds according to the following equation:



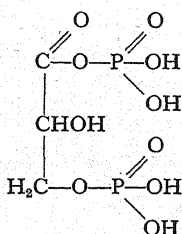
The quaternary nitrogen of thiamine presumably accepts the two electrons first; a yellow enzyme (alloxazine-adenine-nucleo-protein Warburg & Christian, 1938); then transfers the electrons to oxygen (Lipmann, 1939).

(3) In the presence of another bacterial enzyme, acetylphosphate (synthetically prepared) reacts with adenylic acid according to the following equation:

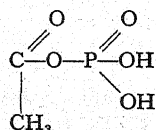


Thus, two types of carboxylphosphate esters have been demonstrated:

(1) 1, 3-diphosphoglyceric acid (phospho-glyceryl-phosphate):



and (2) acetylphosphate (phosphorylacetate):

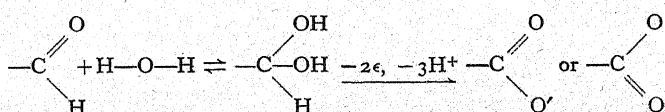


IV. PHOSPHATE AND REVERSIBILITY

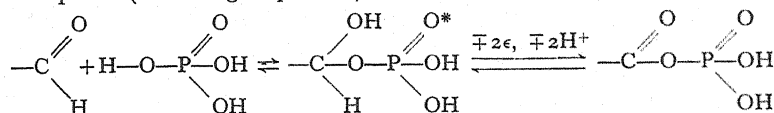
The essential phenomenon in both carbonyl oxidations is, as pointed out by Lipmann (1939), that phosphate replaces water in the formation of the electron donor proper.

A comparison of the two types of oxidation is very illuminating. The pyridine-nucleotide is electron acceptor.

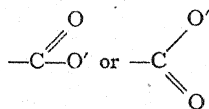
(1) Water (Wieland-Thunberg):



(2) Phosphate (Warburg-Lipmann):

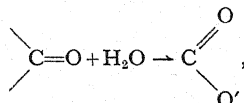


Type (1) is an oxidation accompanied by a large liberation of free energy, i.e. a large increase in stability corresponding to the formation of a resonating structure



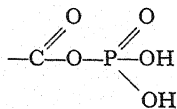
(Lewis, 1923).

The large decrease in free energy accompanying this type of carbonyl oxidation, in Clark's terminology expressed as a very negative redoxpotential of the system,



appears very clearly from thermal data (Parks & Huffmann, 1932); oxidations of this kind are considerably more exergonic than the oxidation of hydrogen gas (Borsook, 1935).

Type (2) is an oxidation where only a very small free-energy change is involved, i.e. practically no increase in stability. In the structure

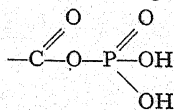


not only the harmonic resonance of carboxylate but also that of phosphate is eliminated.

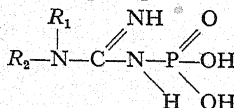
This simultaneous elimination of two resonating structures will be referred to here as opposing resonance (C. D. Coryell, private communication).

Opposing resonance which also occurs in acetic acid anhydride is known to cause a decrease of stability of about 10,000 cal. (i.e. increase the free energy by the same amount). Acetic acid anhydride, acetylphosphate and glycerylphosphate all exhibit an absorption line in the ultraviolet, $m\mu$ 217 (Negelein & Brömel, 1939; Lynen, 1940).

Opposing resonance occurs in the following biologically important structures:

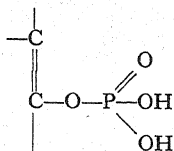


carboxylphosphate (phospho-glyceryl-phosphate, acetyl-phosphate),

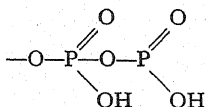


guanidinephosphate (creatinephosphate (phosphocreatine), argininephosphate),

* The carbonyl group probably takes up phosphate independently of any enzyme.



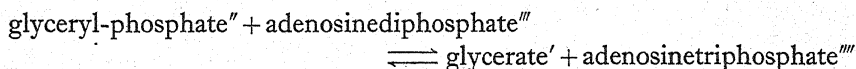
enol-phosphate (phospho-enol-pyruvate),



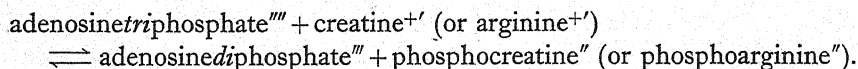
pyrophosphate (adenosinediphosphate, adenosinetriphosphate, thiaminepyrophosphate, pyridine- and alloxazine-dinucleotides).

It has been emphasized earlier that all these related structures have the same thermodynamical property, giving rise to a liberation of about 10,000 cal. when hydrolysed. Ordinary hydroxyphosphate linkages (glycerophosphate, hexose-phosphates) liberate not more than 1000-2000 cal. when hydrolysed. The reason for the high liberation of free energy when the acid anhydride structures are hydrolysed is evident. The acid anhydride structure has a low stability (opposing resonance), whereas the products of hydrolysis, free acids, possess an unusually high stability (resonance). The hydrolysis of acid anhydrides therefore gives a very large increase in stability or a large fall in free energy. The same applies to the splitting of guanidinephosphate to the resonating structures guanidine (creatine, arginine (Pauling, 1939)) and phosphate.

We are now in a position to understand the reversible reactions:



and

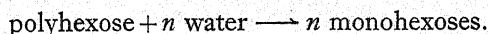


The energy of the carboxylphosphate is stored in the related pyrophosphate or guanidinephosphate structures.

Thus, the oxidation of carbonyl structures coupled with phosphate uptake is able to transform the high energy of the carbonyl structure to pyrophosphate or guanidinephosphate energy with a very small loss of energy.

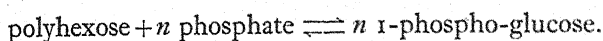
The replacement of water by phosphate converting practically irreversible reactions into easily reversible reactions is a feature not only confined to oxidation reduction.

Polyhexoses (starch, glycogen) are broken down to monohexoses in two different ways. Enzymes occurring in the digestive tract hydrolyse polyhexoses according to the equation



The other mechanism by which polyhexoses are broken down takes place in liver and muscles and has been revealed by the brilliant studies of Cori & Cori (Cori & Cori, 1936; Cori *et al.* 1937; Cori *et al.* 1939) and of Kiessling (1939).

The splitting which is not a hydrolysis but a phosphorolysis is reversible and proceeds according to the equation:



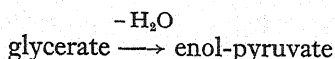
The 1-phospho-glucose (Cori-ester) was discovered in 1936 by Cori & Cori, who also obtained this ester by purely chemical synthesis (1937). A specific enzyme converts the 1-ester into 6-ester.

The discovery of the carboxylphosphate has not only been able to give us the first purely chemical explanation of the coupling between an oxidation and an assimilation (synthesis), but does also explain Meyerhof's discovery that the reduction of phosphoglycerate to phosphoglyceraldehyde (by reduced pyridine-nucleotide) requires dephosphorylation of adenylypyrophosphate. In the light of the new discoveries this has the following interpretation: the stable (resonating) carboxylate ion cannot be converted into the much less stable carbonyl structure unless either an extremely exergonic ('negative') redox system serves as electron donor, or the stable carboxylate structure is converted, by another energy donating system, into a carboxyl ester of much less stability. Biological systems hardly possess redox systems 'negative' enough to overcome a reduction of the stable carboxylate ion on a larger scale. Biological systems, however, possess another energy donating system powerful enough to overcome the stability of carboxylate ion; this energy donor is the pyrophosphate structure which we find in Lohmann's adenylypyrophosphate (adenosinetriphosphate). We know that this energy donor is used at least in the reduction of phosphoglyceric acid to phosphoglyceraldehyde. In the living cell, where adenylypyrophosphate occurs in very limited amount, the pyrophosphate utilized for endergonic reductions is restored by oxidations, in animal tissues by respiration, of lactate, pyruvate or other metabolites. The necessity for oxygen when lactate is converted to sugar is a very old observation. It is possible that also lactate and malate oxidations involve phosphate uptake; in a number of cases, however, we have not yet a real understanding of the coupling between respiration and phosphorylation (Colowick *et al.* 1940).

The thermodynamical significance of the phospho-enol-pyruvic acid of Lohmann, Meyerhof & Kiessling was first recognized when Meyerhof and collaborators showed that this ester was able to phosphorylate adenylic acid to adenylypyrophosphate, and that the mineralization of the enol-ester to ketopyruvic acid and inorganic phosphate released a considerable amount of heat.

The potential energy contained in the enolic ester and in the polyalcohol structure of the phosphoglyceric acid which is in an easily reversed equilibrium with the enolic ester has been calculated most recently by Lipmann (1941). He estimates the ΔF of the reaction: phospho-enol-pyruvic acid \longrightarrow ketopyruvic acid + phosphate to be about $-11,250$ cal. per mol. P.

Lipmann stresses the analogy to the oxidation of carbonylphosphate; in this case the strongly exergonic anhydride reaction



is counterbalanced by the formation of an energy-rich ester bond, enol-phosphate.

Meyerhof *et al.* (1938*a*) have shown that adenylypyrophosphate is unable to phosphorylate a measurable amount of pyruvic acid to phosphopyruvic acid. The potential energy of the polyalcohol is apparently higher than that of the pyrophosphate. This means that the polyalcohol structure of sugar cannot be reconstructed directly by pyrophosphate energy as is the case with the reconstruction of the carbonyl structure.

The important studies of sugar formation with radioactive lactic acid $\text{CH}_3\text{—CHOH—COOH}$ as tracer (Conant *et al.* 1941) indicate that lactate is converted to sugar by an oxidation in which the carboxyl group is lost as carbon dioxide.

Lipmann (1941) has suggested that 2 mol. of lactate are oxidized through the dicarboxylic system. It is known (Kalckar, 1937) that fumaric acid can be oxidized to phospho-enol-pyruvic acid. By this mechanism 2 mol. lactate would be required for the formation of 1 mol. triose. Since the oxidative steps from pyruvic acid to fumaric acid would be available for phosphorylation of adenine-nucleotides, an oxidation of other metabolites as for instance sugar would be decreased correspondingly.

V. PYROPHOSPHATE ENERGY AND CHARGING

Is pyrophosphate energy used in other assimilatory processes?

Adenylypyrophosphate occurs in a relatively large amount in muscle tissue. It is in equilibrium with phosphocreatine which occurs in three times as large amount as adenylypyrophosphate. During muscular work the guanidinephosphate is utilized and inorganic phosphate increases; the adenylypyrophosphate remains unchanged. The guanidinephosphate is restored by oxidations (fermentation or respiration). Lundsgaard observed that in iodoacetate-poisoned muscle, where all oxidations are stopped, the phosphocreatine is consumed to a much larger extent than in normal muscles; when all phosphocreatine is consumed, the adenylypyrophosphate fraction decreases. As soon as this fraction is consumed, the muscle is exhausted in a stage of rigor, i.e. all relaxation of the contracted myosin is stopped. Perhaps the free energy of the pyrophosphate structure which is liberated in the dephosphorylation process can be utilized in the relaxation of the discharged system. This idea was pointed out by D. M. Needham (1937), by Lundsgaard (1938), and by later investigators. The large amount of free energy released in the hydrolysis of pyrophosphate is hardly scattered as heat, as would be the case if a plain hydrolysis took place. The pyrophosphate energy could be utilized only when the dephosphorylation of this group is coupled in some way or other with changes in the

myosin system. A phosphorylation of guanidino groups in the contracted myosin might start the relaxation process during which the phosphate is liberated as inorganic phosphate.¹

Do we have any experimental basis for the assumption that a coupling between pyrophosphate hydrolysis and myosin contraction exists? So far nobody has provided direct evidence for such a coupling.

A reaction between a phosphate anhydride and basic groups in proteins has been clearly demonstrated. Perlmann (1938) showed that metaphosphate forms dissociable, in some cases crystalline, compounds with a number of proteins. The compound dissociates on the alkaline side of the isoelectric point of the protein. The proteins remain in the native state.

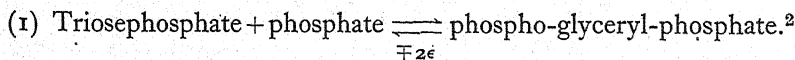
A reaction between adenylypyrophosphate and one of the muscle proteins is very possible. Engelhardt & Ljubimova (1939) recently observed that adenylypyrophosphatase, the enzyme which catalyses the hydrolysis of the pyrophosphate group, occurs in large amounts in the myosin fraction.

An increasing number of biochemists share the view that in the living cell adenylypyrophosphate is not dephosphorylated directly but through cellular structures, acting as a kind of phosphate transfer system.

The birefringence of isolated myosin has been investigated recently by J. Needham *et al.* (1941). The birefringence of isolated myosin is easily changed, for instance by altering pH and salt concentration. The investigators found a marked and very rapid decrease of birefringence when adenylypyrophosphate was added to the system. The birefringence slowly increased again. These recent observations might indicate a more or less direct reaction between adenylypyrophosphate and myosin.

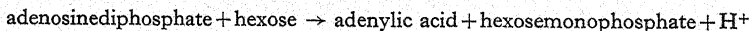
If we suppose that myosin in the contracted state acts as a phosphate acceptor and during the relaxation process as a phosphate donor, then we would get an illustration of how changes in cellular structures are able to 'regulate' metabolic processes.

The coupled reactions might be illustrated as follows:



¹ The transfer of phosphate from adenylypyrophosphates (either adenosinetriphosphate or -diphosphate) to glucose or fructose yielding adenylic acid and hexosemono- or diphosphate does also release a great amount of free energy. Recent experiments by Colowick & Kalckar (1941) indicate that the reaction between adenylypyrophosphate and sugar is complex.

It was found that the reaction:



requires, beside the ordinary enzyme (hexokinase) and Mg^{++} , the presence of an acid-stable protein which was found only in muscle tissue and therefore called myokinase. Myokinase is active in amounts less than 1 μg . protein per c.c.; it is precipitated by trichloroacetic acid but is easily redissolved at neutral reaction and shows full activity. Myokinase is inhibited by adenylic acid; this and other observations might indicate that myokinase acts as a phosphate transfer system. A direct demonstration of a phosphorylation of myokinase has so far not been possible.

² Pyridine-nucleotide is electron acceptor.

- (2) Phospho-glyceryl-phosphate'' + adenosinediphosphate'''
 \rightleftharpoons phosphoglycerate' + adenosinetriphosphate'''.
 (3) Adenosinetriphosphate''' + contracted myosin
 transformation of chemical
 into mechanical energy
 \longrightarrow adenosinediphosphate''' + phosphate'' + relaxed myosin.
 $-\Delta F \text{ large}$
 (4) Relaxed myosin \longrightarrow contracted myosin.

The more contracted myosin the more consumption of adenine polyphosphates and the more oxidation of triosephosphate. Thus, according to these considerations, a contraction of myosin starts the oxidation of phosphotriose to pyruvate and of the latter to the acetate level.

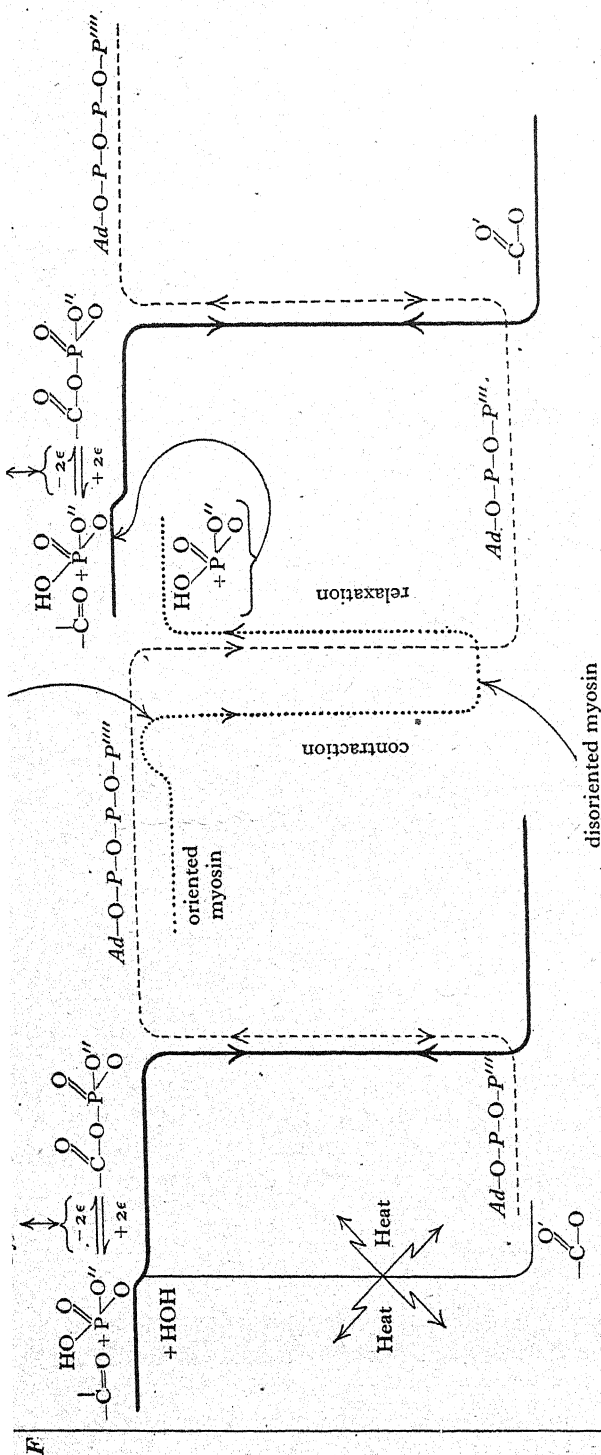
The sudden increase in cellular respiration (or, in the absence of oxygen, in lactic acid formation) succeeding a muscle contraction is an old observation. Recent studies of Millikan (1937) who measured the rate of reduction of myoglobin in rest and during contraction show that the increase in oxygen consumption appears less than $\frac{1}{5}$ sec. after the contraction starts.

The dephosphorylation of creatinephosphate, the other reaction which rephosphorylates adenylic acid, appears much later than the oxygen consumption. It must, however, be borne in mind that a sensitive method of measuring creatinephosphate hydrolysis, corresponding to Millikan's method of estimation of the rate of the oxygen consumption, does not exist.

In Fig. 1 an attempt is made to describe in chemical terms the well-known biological phenomenon that changes in cellular structures, as for example the contraction and relaxation of myosin, are able to regulate the rate of oxidations.

If carbonyl groups were oxidized according to the old Wieland scheme (i.e. by uptake of water, forming carbonylhydrates which then were oxidized to the stable carboxylate structure), the free energy would be liberated already during the oxidation and therefore scattered as heat. Such a reaction has a very high degree of irreversibility (Lewis & Randall, 1923) and the rate of such a kind of oxidation must therefore remain unaffected by changes in cellular structures.

If a reaction between adenylypyrophosphate and myosin really occurs, a demonstration of such a reaction, using purified myosin as phosphate acceptor, should be possible. However, even if we succeeded in such a demonstration, our understanding of how chemical energy can be transformed into mechanical energy (i.e. the relaxation process) would still be far off. Our understanding of the mechanical phenomenon depends on our knowledge not only of the myosin structure but also of the structures which are connected with the myosin filaments in the intact muscle. What keeps the myosin system relaxed (stabilization of the charged state)? Where does the plate stimulus primarily act? What structure reorients (recharges) the contracted (and perhaps phosphorylated) myosin? It is not unlikely that the same structure which is involved in the stabilization of the charged myosin is inactivated by the stimulus, thus causing contraction of myosin as a secondary reaction. Perhaps the stabilizer system is phosphorylated and thus enabled to



Time

Fig. 1. This diagram shows how metabolism and changes in myosin might be coupled. The balance shows only that when myosin in the muscle contracts and relaxes, carbonyl groups are oxidized. The single steps in this cellular coupling can be detected only by purification and separation

of enzymes. >C=O represents the carbonyl group of sugars or pyruvic acid; —C=O , the carboxylate ion of sugar acids, or lower fatty acids;

—C—O—P(=O)(O)—O— , the carboxyl phosphate of glyceryl phosphate or acetyl phosphate; Ad—O—P—O—P''' , adenosine diphosphate, and $\text{Ad—O—P—O—P—O—P—O—P'''}$, adenosine triphosphate (the number of negative charges is in accordance with Lohmann); F , free energy (the relative changes in free energy are arbitrary). The continuous lines represent energy of metabolite, the dashed line represents energy of adenine polyposphate, the dotted line energy of myosin. The electrons ($\pm 2e$) are accepted by the pyridine-nucleotide or furnished by the reduced pyridine-nucleotide. The biological carbonyl oxidations seem mainly to follow the pathway of phosphate uptake and not of water uptake. This fact is indicated in the figure by the thick line illustrating the first type of oxidation and the thin line illustrating the second type. The 'bump' in the myosin curve at the time of stimulus indicates the 'wall of potential' which prevents the spontaneous discharge (contraction) of myosin.

reorient the discharged myosin. All these assumptions are so far pure speculations. However, some of these problems might very well be answered within the next ten years.

The studies of single muscle cells (Weber; Buchthal) as well as of isolated myosin (Edsall; Needham *et al.*) will probably be of much importance for our understanding of these problems.

VI. CONCLUSION

The phosphate esterified to a number of important electron donors and transfer substances is essential for the binding of these systems to catalytically active specific proteins (enzymes) which, by combining with their specific substrates, depress the potential barrier between the electron-saturated substrate and the reactive substrate proper, the free radical (cf. Michaelis & Smythe, 1938). The phosphate, esterified to the substrate, combines presumably with the basic groups in the catalytically active protein.

Typical example: phosphoglyceraldehyde needs 1000 times smaller amounts of enzyme than free glyceraldehyde in order to be oxidized to the corresponding acid (Warburg & Christian, 1939; Negelein & Brömel, 1939). Inorganic phosphate combines with carbonyl groups, replacing the corresponding reaction with water; the carbonylphosphate is enzymatically oxidized (by pyridine-nucleotides) to carboxylphosphate, a structure which unlike carboxylate still keeps most of the free energy of the carbonyl group. The carboxylphosphate donates the phosphate to adenine-nucleotides, forming pyrophosphate; since this reaction is easily reversed the energy of the original carbonyl group is still preserved, stored in the pyrophosphate structure. The adenylypyrophosphate is believed to be able to react with specific cellular structures.

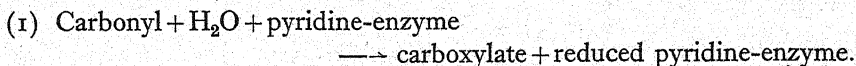
Carbonyl oxidation with water releases the energy actually during the oxidation, carbonyl oxidation with phosphate preserves the energy until it is transferred to certain discharged systems. A part of this energy transfer has been revealed.

In the degradation of the polyhydroxystructure to the α -keto acid structure the scattering of energy is prevented by the other phosphate group. The anhydride of 2-phosphoglycerate, phospho-enol-pyruvate, is converted into keto-pyruvate with simultaneous formation of a pyrophosphate linkage.

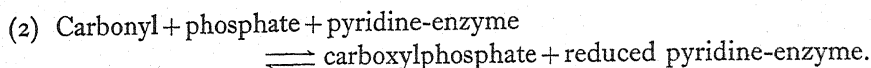
Thus the phosphorylation prevents the high potential energy of the aldehyde group as well as that of the polyalcohol part of the sugar molecule from scattering.

VII. SUMMARY

Two different types of carbonyl oxidation exist:



This type of oxidation is practically irreversible and consequently liberates a large amount of free energy. The normal redoxpotential of this system is extraordinarily low, i.e. carbonyl is a very strong reducing agent.



This type of oxidation is reversible and the change in free energy accordingly very small, which means that the normal redoxpotential of the system: carbonyl + phosphate \rightleftharpoons carboxylphosphate + $2e + 2H^+$ is very close to that of the system: pyridine-enzyme \rightleftharpoons reduced pyridine-enzyme + $2e + H^+$.

Thus replacement of water by phosphate in the carbonyl oxidation eliminates a great fall in free energy by transforming the labile carbonyl into an equally labile acid anhydride, carboxylphosphate.

The degradation of the polyalcohol structure of the sugar acid (glyceric acid) to the α -keto acid structure of pyruvic acid releases a considerable amount of energy.

Moreover, in this case the energy is directed and preserved by means of a phosphorylation.

I am indebted to Dr D. M. Needham (Cambridge) for her generous help in preparing the manuscript for publication and to Prof. C. F. Cori, Dr G. T. Cori and Mr S. P. Colowick for their valuable and helpful suggestions.

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THE EVOLUTION OF SEX IN FLOWERING PLANTS

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I. INTRODUCTION

FLOWERING plants are commonly *hermaphrodite*, having both sexes in the same flower, but in some species the sexes are separated. A species which has separate male and female flowers on the same individual is *monoecious*, and a species consisting of completely male and completely female individuals is *dioecious* (unisexual). To save confusion, the terms hermaphrodite, monoecious and dioecious as defined above will be used exclusively in this article.

In plants dioecy can be seen to have arisen repeatedly from hermaphroditism. The view that dioecy in animals has arisen originally from hermaphrodite or sexually non-differentiated types is held by Darlington (1939). Sexual differentiation by chromosome segregation must follow similar lines whether it is from hermaphrodites or sexually non-differentiated ancestors. For this reason a legitimate comparison between sex mechanisms in plants and animals can be made.

The distribution and frequency of sexual types in the families of flowering plants give valuable evidence for the evolution of sex-determining mechanisms, and since it supports the genetical evidence it serves as a useful introduction to the problems involved. Dioecious plants are rather rare, but widespread in their occurrence throughout the families of flowering plants. This rare but widespread occurrence of dioecy was pointed out by Yampolsky (1922) in a general survey of sex forms in the Phanerogams, compiled from Engler and Prantl's *Natürliche Pflanzenfamilien*. On the one hand, more than 70% of the genera are wholly hermaphrodite and only 5% wholly dioecious. Yet on the other hand, 75% of the

families have some dioecious species. The material is so extensive, however, that the sex form of a number of species is only imperfectly known. For these reasons a survey of the distribution of sexual types in a small and well-known flora, such as the British flora, is what is needed first.

The occurrence of the three main sex types in the British flora is analysed in Table 1. More than 92 % of the species are said to be typically hermaphrodite with perfect flowers; 5.5 % are monoecious, while only 2 % are dioecious. Nevertheless, the 2 % dioecious species, fifty-four in number, are distributed over twenty-six genera in eighteen families, ranging from the Salicaceae to the Compositae.

The sporadic occurrence of unisexuality and the presence of dioecious species in advanced orders shows that it has evolved from hermaphroditism. In fact, the presence of a single dioecious species in an otherwise hermaphrodite genus, which so frequently occurs, indicates that the change to dioecy must be quite recent. It is true that certain families, such as the Salicaceae, are entirely dioecious, but such

Table 1. *Species of the British flora arranged to show the frequency and distribution of sex forms in genera and families (based on the London Catalogue and Bentham and Hooker)*

	Families	Genera	Species	
Completely hermaphrodite	63	468	2080	92 %
Completely monoecious	6	28	122	5.4 %
Completely dioecious	6	15	54	2 %
Hermaphrodite + monoecious	4	1	—	—
Hermaphrodite + dioecious	7	9	—	—
Monoecious + dioecious	2	2	—	—
Hermaphrodite + monoecious + dioecious	3	—	—	—
Total	91	523	2256 (approx.)	

a degree of success is rare: the other five dioecious families in the British flora are all small ones, containing one species each. Taking all the facts into consideration, viz. the sporadic occurrence, the presence in both high and low orders, and the vestiges of the other sex organs in flowers of dioecious species, there is little doubt that dioecy is a development from hermaphroditism. This, however, does not imply that the flowering plants are tending towards unisexuality, but rather that unisexuality is a short-lived condition which meets the special genetic requirements of outbreeding (cf. Mather, 1940). Again, the absence of scattered hermaphrodite species in large dioecious groups indicates the irreversibility of long-term dioecism.

Reference to Table 1 shows that the monoecious condition is almost twice as common as dioecy, but it is still much rarer than hermaphroditism. There are 122 species (5.4 %) which are monoecious. These species are distributed in thirty-one genera and fifteen families. In view of the wide distribution and the fact that most monoecious flowers have the rudiments of the opposite sex, we can conclude that monoecious plants have evolved from perfect-flowered hermaphrodites.

Since it is evident that both the monoecious and dioecious conditions have evolved from hermaphroditism, it is important to determine whether the monoecious

condition is a frequent transitional stage in the evolution of unisexuality. Taxonomic evidence on this problem can be obtained by examining the sex condition in species which are closely related to dioecious species. This type of evidence does not preclude the possibility of parallel evolution of monoecy and dioecy from hermaphroditism. Such evolution is rare, but it may occur in the Compositae, in which perfect-flowered (hermaphrodite) species predominate, but in which both monoecious and dioecious species occur. The purely dioecious genus *Antennaria* only differs from the monoecious genus *Gnaphalium* in the sex form of the flowers, and at one time was included in the same genus. It is possible that the dioecious condition of *Antennaria* arose from a monoecious form similar to *Gnaphalium*. However, in collecting the data *Antennaria* and similar types have been classified as doubtful. Most other dioecious species are easily classified in this respect. For example, *Lychnis dioica* in the Caryophyllaceae, and *Rumex Acetosa* in the Polygonaceae, have probably arisen from hermaphrodite forms, since no monoecious species occur in these families. On the contrary, *Bryonia dioica*, *Mercurialis annua* and *perennis*, and *Carex dioica* are in orders which contain no hermaphrodite species, and it is to be assumed that, in these cases, unisexuality has evolved from the monoecious

Table 2. *Classification of the genera of the British flora according to the sex forms of the species*

	Remaining species are	
	Monoecious	Hermaphrodite
Genera with a majority of dioecious species	None	None
Genera with a minority of dioecious species	17	9
Genera with no dioecious species	29	488

condition. When a whole family, such as the Salicaceae, is completely dioecious, then related families may be considered. The results of this type of classification for the British flora are summarized in Table 2, in which it is evident that the dioecious condition is much more frequently associated with monoecy than with hermaphroditism. The data for the flowering plants in general (Yampolsky, 1922) agree with those of the British flora. There are 172 genera with both dioecious and monoecious species, with or without hermaphrodites, while only twenty-five genera have dioecious and hermaphrodite species without monoecious species. The association of dioecy with the monoecious condition is therefore of general occurrence in the flowering plants.

Further evidence that an examination of the sex forms of related species gives a true indication of the sexual type from which a dioecious species has evolved arises as follows. Dioecious species occasionally return to the monoecious or hermaphrodite condition. Examples of dioecious species occur related to hermaphrodites and having hermaphrodite intersex¹ variants. Examples of dioecious species occur related to monoecious species, and having monoecious intersex variants. A third type have intersex variants which are usually monoecious but

¹ An intermediate between the two normal sexes in a sexually differentiated species.

which occasionally produce some hermaphrodite flowers. These three types are given in Table 3. Thus in every case the type of aberration occurring within the species corresponds with the condition of the species related to it.

Table 3. *Dioecious species classified according to the sexual condition of species related to them and of the variants within the species*

Related to hermaphrodites	Related to monoecious species	Related to monoecious species
Hermaphrodite variants	Monoecious variants	Monoecious variants with occasional hermaphrodite flowers
<i>Rumex Acetosa</i> (Ono, 1930, 1935)	<i>Empetrum nigrum</i> (Hagerup, 1927)	<i>Mercurialis annua</i> (Yampolsky, 1925)
<i>Lychnis dioica</i> (Shull, 1911)	—	<i>Morus alba</i> (Schaffner, 1929)
<i>Fragaria elatior</i> (Valleau, 1923)	—	<i>Salix</i> hybrids (Heribert- Nilsson, 1918)
—	—	<i>Myrica Gale</i> (Davey & Gibson, 1917)

From the taxonomic evidence we can infer that the dioecious condition has arisen from both hermaphroditism and monoecy, and that the dioecious condition is more frequently associated with monoecy than with hermaphroditism.

II. EXPERIMENTAL SYNTHESIS

(1) *Synthesis of dioecious strains*

Abortion of the sex organs determined by genes is frequently found in flowering plants; this abortion is distinct from the type of partial sterility which is due to hybridity. Some genes suppress both sex organs, others affect the male or female organs only. It is the genes which are specific to one sex in their action with which we are concerned. In Table 4 they are classified according to the sex suppressed and to the dominance relationship with the normal gene. It is evident that male organs abort more commonly than female, and that a dominant gene causing abortion is rare.

By making suitable combinations of such genes, Jones (1932, 1934) and Emerson (1932) have been able to produce dioecious strains of *Zea Mays*, a plant which is normally monoecious. Jones used the recessive mutant gene 'silkleless', *sk*, which suppresses the silks (pistils) of the female flowers, and the recessive mutant gene 'tassel seed two', *ts2*, which suppresses the anthers and causes the development of female organs in the male flowers. The gene *sk*, however, is not effective in the presence of homozygous *ts2*, so that a female plant *sk sk ts2 ts2* has a normal female inflorescence while the male inflorescence is converted to female. The female plant of this strain is thus homozygous for both the mutants. The male is *sk sk Ts2 ts2*, *sk* having caused the female flowers to abort and *ts2* being unable to turn the males into females because of the presence of its wild-type allelomorph. In this strain the male is thus the heterogametic sex.

Emerson synthesized two dioecious strains of maize; in one of these the female was the heterogametic sex, in the other the male. For the suppression of the female inflorescence the gene 'barren stalk' *ba*, was used instead of *sk*, to which it is

similar in action. For the male heterogametic strain the gene *ts2* was used, for the female heterogametic strain a dominant mutant, *Ts3*. In action these genes are similar, but *ts2* is recessive and *Ts3* dominant. Thus *ts2 ts2* is female while *ts3 ts3* is male. The genetic constitutions of Emerson's strains are:

Male heterogametic sex: ♀, *ba ba ts2 ts2*; ♂, *ba ba Ts2 ts2*.

Female heterogametic sex: ♀, *ba ba Ts3 ts3*; ♂, *ba ba ts3 ts3*.

The strain with a male heterogametic sex is comparable with that of Jones. In the strain with a female heterogametic sex, *ba ba Ts3 ts3* is female because *Ts3* has

Table 4. *The occurrence in flowering plants of male and female sterility caused by recessive or dominant genes*

Male sterility				Female sterility			
Recessive	No. of different genes	Dominant	No. of different genes	Recessive	No. of different genes	Dominant	No. of different genes
<i>Zea Mays</i> (Beadle, 1932)	15	<i>Zea Mays</i> (Jones, 1934)	3	<i>Zea Mays</i> (Emerson, 1932)	2	—	—
<i>Oryza sativa</i> (Nagai, 1926a)	1	—	—	<i>Oryza sativa</i> (Nagai, 1926b)	1	—	—
<i>Sorghum</i> sp. (Karp & Stephens, 1936)	1	—	—	<i>Phleum pratense</i> (Witte, 1919)	1	—	—
<i>Lathyrus odoratus</i> (Bateson et al. 1905)	1	—	—	<i>Pharbitis nil</i> (Imai, 1926)	1	—	—
<i>Lycopersicum esculentum</i> (Crane, 1915)	1	—	—	<i>Rubus idaeus</i> (Crane & Lawrence, 1931)	1	—	—
<i>Antirrhinum</i> sp. (Baur, 1924)	4	—	—	<i>Pisum sativum</i> (Sverdrup, 1927)	1	—	—
<i>Rubus idaeus</i> (Crane & Lawrence, 1931)	1	—	—				
<i>Prunus persica</i> (Connors, 1926)	1	—	—				
<i>Oenothera organensis</i> (Emerson, 1938)	1	—	—				
Total	26		3		7		0

turned the male flowers into females while *ba* was not able to reverse the sex of the male flowers in the presence of *Ts3*, and *ba ba ts3 ts3* is male because the homozygous recessive wild-type *ts3 ts3* has no sex-reversing action, whereas *ba* turns the female flowers into males.

There are three important points emerging from these experiments:

- (1) The sex-determining mechanism is the segregation of a single gene.
- (2) Other genes in other chromosomes modify the expression of this gene.
- (3) Male and female heterogametic races have been produced.

Another example of experimentally produced dioecy is in *Rubus idaeus*. This plant is normally hermaphrodite. A carpel suppressor gene, *f*, and an anther

suppressor gene, *m*, have been found (Crane & Lawrence, 1931), and although a true-breeding dioecious strain has not been produced the species shows a possible early stage in the evolution of dioecy in a perfect-flowered (i.e. hermaphrodite) plant. The four sexual types and their genetic constitution, which results from the interaction of the two genes, are given below:

Sex	Genetic constitution	Leaf type
♂	<i>M F</i>	Normal: 5 and 3 lobed
♀	<i>mm F</i>	Normal: 5 and 3 lobed
♂	<i>M ff</i>	Obtuse: 3 and 1 lobed
Neuter	<i>mm ff</i>	Obtuse: 3 and 1 lobed

The obtuse leaf of the males and neuters is another expression of the carpal suppressor gene; the vegetative shoots of the hermaphrodite and female have five-lobed leaves and the flowering shoots three-lobed leaves, whereas in the male and neuter the leaves are three-lobed on the vegetative shoots and single-lobed on the flowering shoots. This leaf dimorphism is significant because in one of the two dioecious species of *Rubus*, viz. *R. Chamaemorus*, there is a similar dimorphism of the leaf.

Since the genes are independent, the result of crossing a female *mmFf* with a male *Mmff* is 1 ♀ : 1 ♀ : 1 ♂ : 1 neuter¹. Therefore the system is not self-perpetuating, and for it to become a true-breeding sex mechanism two changes are necessary: a shift of dominance of one of the genes with respect to the normal allelomorph and complete linkage. The shift of dominance ensures that only one sex is heterogametic; thus if the carpal suppressor gene *f* becomes dominant, then the male is $\frac{MF}{mf}$ and the female $\frac{mf}{mf}$ instead of male $\frac{Mf}{mf}$ and female $\frac{mF}{mf}$. The complete linkage prevents the production of *Mf* and *mF* gametes which would give rise to hermaphrodites and neuters.

Dominance modification could be effected by selection of modifying genes or allelomorphs, complete linkage by a structural chromosome change such as an inversion, or by both the genes being situated close together in the same chromosome (Darlington, 1939). Which sex will be heterogametic depends on which gene develops a shift towards the dominant condition. If dominance of the anther suppressor gene is selected, then the female will be the heterogametic sex; conversely, if the carpal suppressor gene becomes dominant, then the male will be heterogametic. A similar situation to that described in *Rubus idaeus* is found in *Oryza sativa* (Nagai, 1926*b*), which is also a perfect-flowered hermaphrodite; but here the interaction of the sterility genes is not known in such detail as in *Rubus*.

Since male and female sterility genes segregate in artificially inbred plants, it may be assumed that similar mutants occur in wild populations (and this is necessary for the evolution of dioecy from hermaphroditism). Few reports of sporadic male and female sterility have been made, but this does not mean that it does not occur. The lack of critical observation and the difficulty of such observation would account

¹ A neuter is an individual without sexual organs, and in this case is recessive for both genes.

for their rarity. However, the sexual forms of *Rubus idaeus* have been reported in the wild, the males are described as var. *obtusifolius*. In *Coptis japonica* (Akemine, 1935) phenotypically similar sex forms are present in about 2% of the wild population. The genetics of sexual polymorphism in this plant is not known, but it is probably similar to the case of *Rubus* and *Oryza*.

The synthetic dioecious strains which have been described show with exactitude the genetic origins of dioecious plants; genes suppressing the male and female organs are always involved. It is important to note that, in contrast to the dioecious strains of monoecious maize, separation of the sexes in hermaphrodite *Rubus* and *Oryza* depends upon the segregation of two genes instead of one. Further, it is necessary for the complete evolution of the dioecious condition that one of these two genes should be recessive and the other partially dominant to the normal allelomorphs, and that the two genes should be completely linked.

From the taxonomic evidence we saw that dioecy is more frequently associated with monoecy than with hermaphroditism. The genetical evidence indicates the reason for this; the change from hermaphroditism to dioecy requires more steps than from monoecy.

(2) *Synthesis of male sterility*

Male sterility of the type just described should not be confused with the male sterility which has become established in wild populations of certain species. The genic sterility, appearing in experimental plants, is of rare occurrence in the wild but *gynodioecious* species, that is, species consisting of hermaphrodites and females may have a high proportion of male-sterile individuals (i.e. females) in the population. Do these species represent an intermediate stage in the evolution of unisexuality? An examination of the inheritance of male sterility in these cases will help to answer this question.

The inheritance of male sterility has been investigated in ten gynodioecious species. In six of these species, females and hermaphrodites produce both types of offspring, but Mendelian ratios characteristic of single gene differences were not obtained and no simple explanation can be given of the results. In the other four species, *Satureia hortensis*, *Cirsium oleraceum* (Correns, 1928), *Origanum vulgare* (Appl, 1929) and *Lolium perenne* (Jenkin, 1931), more straightforward results were obtained. Female plants, which of necessity are pollinated by hermaphrodites, produce only female progeny; hermaphrodites produce only hermaphrodites. These results cannot be explained by a single gene difference. An explanation, first advanced by Wettstein (1924), is that in these cases male sterility is cytoplasmically inherited. Something is inherited from the female line only; thus it has to be something outside the nucleus and is covered by the terms cytoplasm and plasmon. An alternative genetic mechanism, involving selective pollen-tube growth and selective fertilization of female gametes, is indeed possible (East, 1934), but it requires several unlikely assumptions. Cytoplasmic inheritance seems to be the most plausible explanation for the cases which have been completely analysed; but this does not imply that other mechanisms of male sterility do not exist in gynodioecious species.

There are in fact indications of other complex types of inheritance, but since they have not been fully analysed they cannot usefully be discussed.

The question now arises: How have gynodioecious species evolved, and why do they show this type of inheritance of male sterility? Direct evidence is not available, but a consideration of the cases of plasmatically inherited male sterility in experimental plants is suggestive.

Cytoplasmically controlled male sterility has arisen in *Zea Mays* (Rhoades, 1933). This is the only known case within a pure species, all others being the result of interspecific hybridization. An important example of male sterility caused by the interaction of genes from one species with cytoplasm from another was found by Michaelis (1937) in *Epilobium* hybrids. The hybrid between *E. luteum* ♀ and *E. hirsutum* ♂ is male sterile. By backcrossing this hybrid to *E. hirsutum* ♂ for thirteen generations, plants with a *hirsutum* nucleus and *luteum* cytoplasm were obtained. These plants were also male sterile like the F_1 , thus showing that the male sterility is the result of the interaction of genes from *hirsutum* with the *luteum* cytoplasm. That these plants really had the nucleus of *hirsutum* and cytoplasm of *luteum* was shown by crossing them with wild *E. luteum* ♂; the progeny were similar to the original *luteum* ♀ × *hirsutum* ♂ and not to the reciprocal hybrid. Here we have a male-sterile form of *E. hirsutum* which differs from the normal hermaphrodite solely in possessing the cytoplasm of *E. luteum*.

Cytoplasmically controlled male-sterile races of *Epilobium hirsutum* have been synthesized not only by interspecific hybridization but also by crosses between geographical races. The importance of this will appear later.

Male sterility in *Linum* was reported by Bateson & Gairdner in 1921. In their cultures of *L. usitatissimum* they found a procumbent form, which they assumed to be a variety of *usitatissimum*. Normal ♀ × procumbent ♂ gave an F_2 of all normal plants, but procumbent ♀ × normal ♂ gave one-quarter male-sterile plants in F_2 . This was interpreted by Chittenden & Pellew (1927) and further substantiated by Gairdner (1929) as due to the interaction between the cytoplasm of the 'procumbent' plant and a recessive gene from *L. usitatissimum*. The recessive gene came from the normal *L. usitatissimum*, because the F_1 is normal and because a three to one ratio of normal to male-sterile plants is obtained in the F_2 . The cytoplasm came from the procumbent plant, because this 3 : 1 ratio is obtained only when the original cross is made with the 'procumbent' plant as the female parent. Now Gajewski (1937) has shown that *L. floccosum* is similar in appearance and in its cytoplasm to the procumbent plant of Bateson & Gairdner, and it is fairly certain that the procumbent plant really was *L. floccosum*. This cannot therefore be regarded as a case of male sterility in a pure species (as originally postulated by Bateson). Further instances of male sterility caused by the interaction of genes from one species with cytoplasm from another are given in Table 5.

Cytoplasmically controlled male sterility is therefore common in gynodioecious species and in artificial interspecific hybrids, but has rarely been found as a mutation within a pure species. The conclusion from this is that naturally occurring male sterility has not evolved from single-gene mutations alone, but is probably the

result of interspecific hybridization or cytoplasmic mutation. Further evidence of this might be obtained from a comparison of the nucleus and cytoplasm of gynodioecious species with those of related species; but no data on the point are yet available.

In considering the origin of gynodioecious species two factors must be remembered: (1) Cytoplasmic differences between species are frequently present whereas cytoplasmic mutations are rare. (2) Interspecific hybrids are frequently sterile while cytoplasmic mutations are not accompanied by genic sterility. The first fact

Table 5. *Interspecific hybrids in the flowering plants which are male sterile as the result of the intersection of genes of one species with the cytoplasm of another*

Male steriles in	Hybrid	Author
F_1	<i>Epilobium luteum</i> ♀ × <i>hirsutum</i> ♂	Michaelis (1937)
F_1	<i>Streptocarpus Wendlandii</i> ♀ × <i>Rexii</i> ♂	Oehlkers (1938)
F_2	<i>S. Comptonii</i> ♀ × <i>Rexii</i> ♂	
F_2	<i>Linum floccosum</i> ♀ × <i>usitatissimum</i> ♂	Bateson & Gairdner (1921), Gajewski (1937)
F_2	<i>Geranium Endressii</i> ♀ × <i>striatum</i> ♂	Sansome & Philp (1932)
F_2	<i>Nicotiana Langsdorffii</i> ♀ × <i>Sanderæ</i> ♂	East (1932)

favours the interspecific origin and the second the cytoplasmic mutation origin. Male steriles from crosses between geographical races would not suffer from genic sterility to the same extent as interspecific hybrids and are a likely method of origin of gynodioecy.

The evolution of the gynodioecious condition from single-gene controlled sterility has not been favoured by natural selection, because an equilibrium between the proportions of hermaphrodites and females in a wild population would be impossible to maintain, for the following reason: if the male sterility is due to a recessive or dominant gene, then the male-sterile type, in order to survive, must be more than twice as fertile on the female side as the hermaphrodite. If p is the proportion of females and $q (= 1 - p)$ the proportion of hermaphrodites in a population, and $f(1 - p)^x$ is the fitness of the female relative to the hermaphrodite, the relationship between p and f , where f is the fertility, is shown to be

$$p = \frac{1}{2} \frac{1}{f(1 - p)^{x-1}},$$

when the male sterility is due to a dominant or recessive gene. From this it is obvious that if $f = 2$ then $p = 0$. Therefore the females must be more than twice as fertile as the hermaphrodites if they are to exist in the population. This very high reproductive value of male sterility is one which is not likely to arise suddenly in nature. Cytoplasmic male sterility does not suffer from this disadvantage, and an equilibrium can exist in the population when the fertility of the female is only slightly above that of the hermaphrodite, for in this case $f(1 - p)^x = 1$ (Lewis, 1941).

The second problem with which we are concerned here is the possibility that dioecy may evolve from the gynodioecious condition in which male sterility is

controlled by the interaction between a gene and a different cytoplasm. In the gynodioecious species there are two types of cytoplasm: \square represents the cytoplasm of the hermaphrodite in which the male sterility gene m is ineffective and \circ is the cytoplasm of the female in which the gene m is operative (see Fig. 1). The one possible way in which the evolution of dioecy from this condition could take place is as follows: a gene mutation which suppresses the female organs occurs; this mutant gene F must be completely linked with the male sterility gene m , thus the female is ff \circ mm as in the gynodioecious species. The male would then be Ff \square Mm , since F suppresses the female organs and mm is inoperative in the \square cytoplasm. When this male is crossed on to the female, all the progeny will have the differentiated cytoplasm \circ of the female; the original \square cytoplasm will be eliminated. But in the \circ cytoplasm the gene m will be operative, thus the males will be neuters. Therefore a mutation of the m gene back to the normal allelomorph M is necessary, the male being Ff \circ Mm . As there is only one type of female parent, all cytoplasmic differences have disappeared by the time the dioecious condition is established.

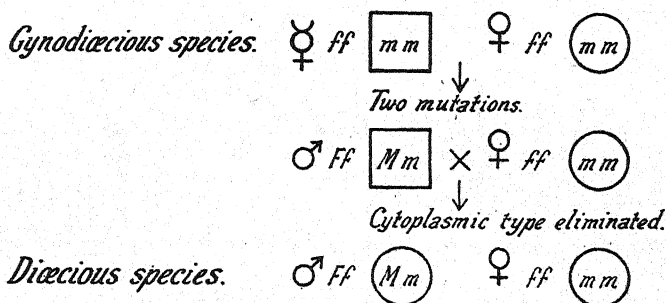


Fig. 1. Steps in the evolution of dioecy from the gynodioecious condition, showing that two mutations and complete linkage of the two mutants are necessary and that the original cytoplasmic difference must be eliminated. \square = original cytoplasm, in which m is inoperative. \circ = differentiated cytoplasm. F = female suppressor. m = male suppressor.

This hypothetical evolution of dioecy involves gene changes which are the same as those required when the evolution is from a hermaphrodite; thus the cytoplasmic differentiation of gynodioecious species is unnecessary for the change and is not a step towards dioecy. Nevertheless, the possibility of gynodioecy being a transitional stage in the evolution of the dioecious condition cannot be ruled out. However, gynodioecy is a stable sexual condition which is adapted for increased outbreeding, because females must be cross-pollinated by hermaphrodites. It is also a more efficient method than dioecy for controlling the degree of outbreeding (cf. Mather, 1940; Lewis, 1941). Furthermore, the taxonomic evidence supports the view that gynodioecy is a separate mechanism and not a step towards dioecy. More than 90% of the gynodioecious species are in the Labiateae; this is a family which contains very few dioecious species.

III. EXPERIMENTAL ANALYSIS

(1) *Analysis of sexuality*

So far we have considered the genetical basis of sexuality in plants which are usually hermaphrodite, and in which dioecious strains have been produced deliberately. It is now desirable to analyse the genetics of sexuality in a plant which is normally dioecious but in which hermaphrodite forms occur. Such a plant is *Vitis vinifera*, which is worth considering in detail, since it is the only normally dioecious species in which a simple genetic sex mechanism has been fully analysed.

The wild European vine, *Vitis vinifera*, is purely dioecious in Central Europe, but hermaphrodites as well as dioecious types have been reported among 'wild' plants in the Rhine valley. It is probable that these hermaphrodites are not truly spontaneous but are escapes from cultivation, since they are found only in areas where vines are largely cultivated. The cultivated varieties of grape are either hermaphrodite or female. Ráthay (1888) names forty-two hermaphrodite and thirty-six female varieties growing at Klosterneuburg. The females of course would require interplanting with hermaphrodites. Since this date therefore it is not

Table 6. *Inheritance of sex in Vitis*

	Progeny
Homozygous ♀ selfed or intercrossed	All ♀
Heterozygous ♀ selfed or intercrossed	3 ♀ : 1 ♂
Homozygous ♀ × female	All ♀
Heterozygous ♀ × female	1 ♀ : 1 ♂
Homozygous ♀ × male	1 ♀ : 1 ♂
Female × male	1 ♀ : 1 ♂
Heterozygous ♀ × male	1 ♀ : 1 ♀ : 2 ♂

surprising to find that there seems to have been artificial selection in favour of hermaphrodites, and female varieties are now much less common. The American species of *Vitis* are strictly dioecious; most of these species produce fertile hybrids when intercrossed or crossed with *V. vinifera*.

The morphological differentiation of the three sex forms is slight. The perfect-flowered form has five erect stamens and a normal pistil. The female flower has the normal number of stamens and a normal pistil, but the stamens are recurved to a position below the base of the ovary. These recurved stamens produce abundant pollen, but the grains are spindle-shaped, have no germ pores and fail to germinate. The male flower has erect stamens and a rudimentary pistil, which contains two carpels each having two abortive ovules. This slight differentiation of the sexes is evidence that the dioecious condition has only recently developed from hermaphroditism.

Genetical studies on the sexuality of *Vitis* have been made by Hedrick & Anthony (1915), Oberle (1938) and other workers, and summarized by Negrul (1936). The results of these workers agree substantially and their main findings are set out in Table 6.

Various interpretations of these results have been made (Valleau, 1916; Rasmuson, 1916; Negrul, 1936; Oberle, 1938). There are two possibilities. The first postulates two completely linked genes, a dominant carpel suppressor *F* and a recessive pollen suppressor *m*. Then the constitutions of the five types would be:

Homozygous ♀	$\frac{Mf}{Mf}$	Heterozygous ♀	$\frac{Mf}{mf}$
Normal ♀	$\frac{mf}{mf}$		
Normal ♂	$\frac{MF}{mf}$	Derived ♂	$\frac{MF}{Mf}$

The second hypothesis is that there are two independent genes, one being epistatic to the other; on this view *f* suppresses the male organs, *M* suppresses the female organs and is epistatic to *F*. The following types are then possible:

Homozygous ♀	<i>FFmm</i>	Heterozygous ♀	<i>Ffmm</i>
Normal ♀	<i>ffmm</i>	Normal ♂	<i>ffMm</i>
Derived ♂ (1)	<i>FfMm</i>	Derived ♂ (2)	<i>FFMm</i>

On this hypothesis there are three possible types of male, and these would behave differently when used for breeding. The data give no indication that these three types exist. One cannot yet say which of the two explanations is the true one.

However, the first hypothesis proposed bears a striking resemblance to the genetic control of sex in *Rubus idaeus*, in which one recessive gene suppresses the male organs and another the female organs. I have shown that a change of dominance of one gene and complete linkage would be necessary for the system to become workable. In *Vitis* these changes may have taken place in the wild populations and dioecious forms replaced the hermaphrodites. That most modern cultivated varieties are hermaphrodite is undoubtedly the result of selection by cultivators for self-fruitfulness. Whether hermaphrodites are the result of a back mutation of the carpel suppressor gene or whether they represent the remnants of the old hermaphrodite ancestor is not clear.

A sex-determining mechanism similar to the one present in *Vitis* is found in *Carica Papaya* (Hofmeyr, 1938) except that homozygous hermaphrodites are not viable. Monoecious forms of *Carica* exist, but the difference between this form and other sexual types has not been investigated.

Mercurialis annua is a dioecious plant which occasionally produces males with a few female flowers and females with a few male flowers. Strasburger (1909) and Yampolsky (1916) on selfing the females found that only females were produced. Yampolsky also claimed that males when selfed produced only male offspring, and explained the inheritance of sex through the cytoplasm. However, Gabe (1939) has recently found that selfed males give three males to one female. Further, he reports that the males can be separated into one abnormal male which breeds true and two normal males which again produce males and females. On this evidence the inheritance of sex is by a single or group of linked genes, and the mechanism is probably similar to that in *Vitis*.

(2) *Analysis of sex chromosomes*

The evolution of sexual differentiation by the segregation of chromosomes follows certain lines which are dictated by the genic requirements of the two sexes and the mechanics of chromosome division. Whether this evolution is from a hermaphrodite, as it usually has been in the flowering plants, or from a sexually non-differentiated type still found in Protozoa, as it may have been in animals, a similar sequence of changes in the chromosomes will take place.

Animals are predominantly dioecious, a condition more suited to their motile habit; plants are predominantly hermaphrodite, a condition more suited to their static existence. Just as the dominant type in animals occasionally gives rise to monoecious species, so the dominant type in the flowering plants occasionally develops into dioecious species. The sporadic appearance of hermaphrodites takes many forms whose unusual character shows how short-lived they must be. For example, in *Icerya purchasi* there are 'hermaphrodites' and males, but the male is in reality a diploid female containing haploid testes; these correspond therefore in genetic constitution with the males (cf. Darlington, 1937).

From non-differentiated types or from hermaphrodites sexual differentiation must have developed, and in this process of development we can legitimately assume a similarity between animals and the flowering plants. Furthermore, every time the system of sexual differentiation is reconstituted the same kind of genetic changes must occur.

In *Drosophila melanogaster* the difference between females and males is determined by a number of genes, and the genes determining feimleness are distributed on a particular chromosome, the X-chromosome as well as to some extent on the fourth (Dobzhansky & Schultz, 1934). The polygenic nature of sex determination as found in *Drosophila* is common to most animals and some plants, but we have seen that in *Zea Mays* sexual differentiation has been produced under experimental conditions by the segregation of one gene. Similarly in the fish *Lebistes*, the segregation of a single gene can differentiate the sexes (Winge, 1932). These sexually simple organisms have no visible differences between sex chromosomes, but in more advanced polygenic types there is such a differentiation of a pair of chromosomes. The differentiation of the sex chromosomes is the result of a series of changes in the chromosomes which meet the peculiar mechanical and genetical requirements of sex segregation (Darlington, 1939). Sex chromosomes are usually of two types, the X and the Y, one sex having XX and the other XY. The X-chromosomes carry the genes determining the homogametic sex and the Y either carries the heterogametic sex-determining genes or is inert. The visible manifestation of inertness in the Y is its variable size in some organisms and complete loss in others. The evolutionary significance of the peculiar behaviour of the Y-chromosome will be discussed after a review of the facts obtained from the study of sex chromosomes in plants and animals.

Many dioecious plants have no distinct sex chromosome, e.g. *Spinacia oleracea* (Haga, 1935), *Shepherdia canadensis* (Cooper, 1932) (cf. Sinoto, 1929). Among plants

with distinct sex chromosomes there is considerable variation in the morphology and constitution of these chromosomes. The simplest type is seen in *Lychnis dioica*, where the female has two equal X 's and the male has one X and one Y (Winge, 1923; Belar, 1925; Blackburn, 1924). Other examples of this XY type of sex mechanism are *Sedum Rhodiola* (Levan, 1933) and *Camnabis sativa* (Nishiyama, 1940). *Fragaria elatior* is similar in having an X - and a Y -chromosome, but in this case the male is XX and the female XY (Kihara, 1930). In all these cases there is presumably a small homologous 'pairing segment' and a larger non-homologous part or 'differential segment' in the X - and Y -chromosomes.

A more complex sex mechanism is the XY^1Y^2 system found in *Rumex Acetosa* (Kihara & Ono, 1925; Sato & Sinoto, 1935; Ono, 1935; Yamamoto, 1938). The Y^1 and Y^2 always pass to the same pole at meiosis and the X to the other pole. The differential segment of the X is median, with two terminal pairing segments, each pairing with a segment of a Y -chromosome. This type of mechanism is derived from two pairs of autosomes by a single translocation and fusion.

In *Humulus Lupulus* two types of sex-chromosome mechanism are found, a simple XX female and XY male type and a derived type with $X^1X^2X^1X^2$ female and $X^1X^2Y^1Y^2$ male (Sinoto, 1929). Here we find that after the XY system had evolved, a structural change took place between the X^1Y^1 and a pair of autosomes (Darlington, 1932).

These three types cover most of the different sex chromosomes in plants; however, mention must be made of the XX female and XO male system in some of the animal groups. This has been derived from the XY system by complete loss of the Y -chromosome.

The action on sex expression of the genes in the sex chromosomes and the autosomes in different plants has been determined by the study of intersex forms of aneuploid (unbalanced), triploid and tetraploid individuals. In *Lychnis*, Warmke & Blakeslee (1939, 1940) showed by the study of triploid and tetraploid plants with different numbers of sex chromosomes that the Y plays a decisive part in sex determination. The normal ratio of X -chromosomes to the number of sets of autosomes, X/A , is one in the female, and even when the ratio is reduced to $\frac{3}{4}$ in a $4A XXX$ plant, the plant is a normal female and not an intersex or hermaphrodite. Evidently in *Lychnis* sex-determining genes are present in the X - and Y -chromosomes and not to any extent in the autosomes. Similar results have been obtained with *Acnida tamariscina* (Murray, 1940).

In *Rumex Acetosa*, unlike *Lychnis*, sex is essentially determined by the balance between the X and the autosomes, the Y -chromosomes being neutral in their action on sex expression (Ono, 1935). The ratio of X -chromosomes to autosomes (X/A) in normal diploid males is $\frac{1}{2}$ and 1 in females. Individuals with a ratio less than a half are males and with greater than 1 are females, and with values between $\frac{1}{2}$ and 1 are intersex forms (see Table 7). Yamamoto (1938) has shown by the production of plants trisomic for different chromosomes that two of the autosomes have a tendency towards femaleness and three of them towards maleness, while the remaining one is indifferent. Therefore in contrast to *Lychnis*, the Y -chromosomes of *Rumex*

Acetosa are mainly inert and the autosomes and the *X*-chromosomes carry the sex-determining genes. This condition is similar to that in *Drosophila melanogaster* (Bridges, 1922).

We are now in a position to formulate the probable sequence of changes in the evolution of sex chromosomes. The gene or two genes which primarily determine sex in dioecious species must be associated sooner or later with a structural change such as an inversion in the chromosomes. A chromosome inversion prevents pairing and hence crossing-over within the inverted segment. This suppression of crossing-over is a condition necessary for the accumulation of groups of sex-differentiating genes, for otherwise the number of sexual types cannot be limited to two (Darlington, 1939). For a single-gene controlled sex mechanism, a structural change is not necessary at the start of the evolution of dioecy from hermaphroditism, though it is likely to be necessary for the further differentiation of the sexes. But when two genes (not yet completely linked) are concerned, as is probably the case in *Vitis*, an inversion is necessary at the beginning to give the necessary linkage of the two genes;

Table 7. *The chromosome constitution and sex of euploid and aneuploid plants of Rumex Acetosa (from Ono, 1935)*

Chromosome constitution		Sex	Ratio $\frac{X}{A}$
Euploids	$X+2A, X+3A, 2X+4A$	♂	$\frac{1}{2}$ or $< \frac{1}{2}$
	$2X+2A, 3X+3A, 4X+4A$	♀	1
	$2X+3A, 3X+4A, 4X+6A$	Intersex	$\frac{2}{3}$ or $\frac{3}{4}$
Aneuploids	$X+2A+a, X+3A+a, 2X+4A+a$	♂	$< \frac{1}{2}$
	$2X+2A+a, 3X+3A+a$	♀ or intersex	Slightly less than 1

unless they are situated close to one another in linkage terms. The inverted region of the chromosomes will develop into the differential segment. In the *X*-chromosome this differential segment can pair and cross-over with the homologous segment on the other *X* in the female, but the differential segment of *Y* never pairs, or at least never crosses over with *X*.

Although a single gene may be sufficient to determine the differentiation of the two sexes of an organism, further genetic differentiation between the sexes, which follows in the course of evolution, can only be effected by the accumulation of more gene differences. The genic requirements of the two sexes will also be entirely different; for example, genes causing abortion of the anthers in *V. vinifera* will be an advantage to the female in which they are superfluous, but a serious handicap to the male in which they are essential. In the fish *Lebistes*, certain completely and partially sex-linked colour and pattern genes are present in the wild males and not in the wild females, but in controlled cultures females can be produced showing some of these genes (Winge, 1927). As Fisher (1931)¹ has pointed out, since these colour genes in the wild are only present in males they must be of selective advantage to the male and not to the female. Because male-favouring genes as a group are

¹ *Biological Reviews*.

bound to be a selective alternative to female-favouring genes, it is essential that these two sets of genes should behave as two discrete units with no crossing-over between them. If crossing-over takes place, intersex forms will be produced. For these reasons a block of male-favouring genes is accumulated by natural selection in the differential segment of the Y-chromosomes and a block of antithetic female-favouring genes in the differential segment of the X-chromosome.

Now this system suffers from a slight disadvantage. This is the lack of recombination between the male-favouring genes in the differential segment of the Y-chromosome. The X-chromosome behaves fairly normally, since the genes in the differential segment can cross-over in the female. The differential segment of the Y-chromosome, which never crosses over, has a different history; it becomes inert

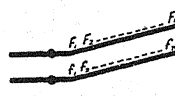
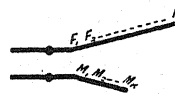
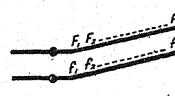
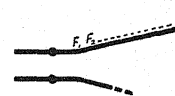
SEX CHROMOSOMES		AUTOSOMES	
	♀	♂	♀ and ♂
A	 <p><i>Free recombination between $F_1 \dots F_K$ Genes</i></p>	 <p><i>No recombination between $F_1 \dots F_K$ and $M_1 \dots M_K$ Genes</i></p>	SEXUALLY INDIFFERENT <i>No recombination between $M_1 \dots M_K$ Genes</i>
B	 <p><i>Free recombination between $F_1 \dots F_K$ Genes</i></p>	 <p><i>No recombination between $F_1 \dots F_K$ and $M_1 \dots M_K$ Genes</i></p>	$M_1 \dots M_K$ Genes <i>Free recombination between $M_1 \dots M_K$ Genes</i>

Fig. 2. Diagram to show the results and significance of the replacement of male determining genes in the Y-chromosome by genes in the autosomes. (*Lychnis dioica* represents type A and *Rumex Acetosa* type B.)

and consequently variable in size. The inertness and consequent ineffectiveness of the Y-chromosome comes about by the gradual replacement of its male-favouring genes by genes in the autosomes. This replacement rectifies the lack of recombination between the genes in the Y-chromosome (Darlington, 1939; see Fig. 2). Thus, if most of the female-favouring genes are in the X-chromosome and most of the male-determining genes are in the autosomes, the requirements of free recombination within the male- and female-determining genes, but no recombination between them, are satisfied. Such a condition has been described in *Rumex* and *Drosophila*, in which sex determination is effected by the balance between X-chromosomes and autosomes and in which the Y is mainly inert.

(3) *Analysis of sex and selection*

Dioecy, like any other inherited character, is subject to the action of natural selection; and to understand the evolution of dioecy the effects of natural selection on the separation of the sexes must be considered.

Dioecy, by necessitating the fusion of gametes from genetically distinct individuals, is an outbreeding mechanism (Darlington, 1939; Mather, 1940). Outbreeding, by increasing the number of heterozygous genes, increases the amount of effective recombination between them. This allows of a more rapid response to the action of natural selection (Fisher, 1930; Muller, 1932; Darlington, 1939). Thus, by making the action of natural selection more efficient, outbreeding itself has a selective advantage. However, this advantage to the species as a whole is only obtained at the expense of the fitness of some individuals, so that the maximum amount of outbreeding is not necessarily the optimum amount (Mather, 1940). Therefore an outbreeding mechanism to survive should be flexible, so that the degree of heterozygosity in a population can be changed fairly rapidly. Dioecy insures that no selfing can take place and that 50% of matings between sibs are ruled out. The degree of outbreeding caused by sexual separation is sometimes considerable, and the amount cannot be changed except by a reversion to hermaphroditism in the case of flowering plants, or by other extraordinary changes such as intra-uterine copulation in *Pediculopsis* (Cooper, 1937). A reversion to hermaphroditism is a difficult process in dioecious plants which have an advanced type of sex-determining mechanism, but it is possible when sex is determined by a few genes. It is significant in this respect that the majority of dioecious plants have a primitive sex mechanism and that hermaphrodites are frequently present.

Other outbreeding mechanisms in plants, such as protandry and protogyny, which probably have a simple selective basis, do not suffer from this disadvantage, and a stable response to the hybridity requirement can be obtained by such means (Mather, 1940). Even the fairly common method of increasing outbreeding by a series of self-sterility allelomorphs can give different degrees of outbreeding. For example, the proportion of sterile sister matings decreases as the number of allelomorphs increases, so that a population containing three allelomorphs will be more outbred than one with a hundred (cf. Wright, 1939). Gene mutations causing self-fertility, i.e. removing the bar to self-fertilization, are not uncommon in self-sterile plants, and these could also afford a respite from rigid outbreeding (cf. Brieger, 1930). Still another defect of unisexuality as an outbreeding mechanism compared with other systems that are available in plants is the wastage of reproductive energy due to indiscriminate dispersal of gametes (Mather, 1940). It is not surprising therefore that dioecy in flowering plants is uncommon and is probably often short-lived. It is a rigid method of outbreeding which has certain defects, and as soon as outbreeding fails to have a selective advantage then the wastage of gametes and the difficulty of returning to hermaphroditism will be a serious handicap.

The wastage of gametes can be mitigated to some extent by changes in the sex

ratio. This ratio varies widely, not only between different species but between different populations of the same species. Usually there is a considerable excess of females over males, only rarely are males found to predominate (cf. Table 8). Now it is evident that in a dioecious plant, the only function of the male is to supply pollen for the fertilization of the female; this is true of the greater number of dioecious animals apart from birds, mammals and the social insects in which the male shows varying degrees of domesticity, and exceptional cases like *Alytes obstetricans* in which the father and not the mother cares for the young. Therefore males in excess of those necessary for the full fertilization of the females will take room which might support productive females. Consequently, sex ratios are variable

Table 8. Sex ratios of dioecious plants

Species	Total population	% males	Author
<i>Silene Otites</i>	8,527	61.9	From Correns (1928)
<i>Spinacia oleracea</i>	54,909	47.7	From Correns (1928)
<i>Mercurialis annua</i>	2,831	46.7	Gabe (1939)
<i>Lychnis alba</i>	10,662	43.8	From Correns (1928)
<i>Cannabis sativa</i>	2,952	41.8	Winge (1923)
<i>Humulus japonicus</i>	428	29.0	Winge (1923)
<i>Rumex Acetosa</i>	5,709	27.6	From Correns (1928)
<i>Humulus Lupulus</i>	1,405	9.8	Winge (1923)
<i>Rumex thyrsoiflorus</i>	6,000	9.5	From Correns (1928)

and presumably suited by the action of natural selection to the reproductive economy of the population. The theoretical sex ratio without discriminate elimination of either gametes or zygotes is 50%. This is also *a priori* the selectively most advantageous ratio (Fisher, 1930). A comparison of sex ratios under artificial conditions and in the wild shows that the elimination is gametal or in the very early zygote stage (cf. Correns, 1928). Further evidence was obtained by Correns in *Lychnis dioica*, *L. alba* and *Rumex Acetosa*, and by Kihara & Hirayoshi (1932) in *Humulus japonicus*, and showed that the elimination occurred while the pollen tubes were growing down the style. Abundant pollination of the stigmas resulted in a low percentage of males in the offspring, while in sparse pollination the males approached 50%. In the case of abundant pollination, competition could take place between the pollen grains, thus affording a means of eliminating either the male- or female-determining grains.

It is possible to outline the mechanism of adjustment of the sex ratio to the reproductive economy. Competition between pollen grains, due to genes controlling the rate of pollen-tube growth, is fairly common in plants (see Mangelsdorf & Jones, 1926; Emerson, 1934; Burnham, 1936; Lewis, 1940), but competition between eggs is less effective. Pollen-tube genes similar to those found in *Zea* and *Rubus* could arise by mutation on the X-chromosome of a dioecious plant. Then an excessive number of male plants in the population will supply the condition necessary for competition between pollen, and consequently fewer Y-carrying than X-carrying pollen grains will fertilize the eggs, thus producing more females in the next genera-

tion. As soon as the number of males in the population is reduced below the optimum, the pollination will be sparse and the competition effect will vanish. In this way there will be a balance struck between the number of males in the population and the reproductive economy. This mechanism can only work if the male is the hybrid sex, and it is significant that the only plant in Table 8 which has an excess of males in the population, namely, *Silene Otites*, is heterogametic in the female sex.

A priori, there is no reason why the female should not be the heterogametic sex as frequently as the male, but in fact only two cases of female hybridity, *Fragaria* and *Silene Otites*, are known, while there are many dioecious plants in which the male is the heterogametic sex (Allen, 1940). Sex mechanisms where the female is the hybrid will often have less chance of survival, since there will be reproductive wastage where the ideal proportion of the sexes is not equality.

IV. SUMMARY

1. The importance of combined genetic and systematic analysis of sex for its evolutionary interpretation has long been understood in animals. It now seems possible to make an evolutionary interpretation of sex in plants by similar methods.

2. From the sporadic distribution of dioecious species throughout mainly hermaphrodite families of flowering plants, it is inferred that dioecious species have evolved from hermaphrodites.

3. From the more frequent association of dioecious species with monoecious species than with hermaphrodites, it is inferred that they have evolved more frequently by means of a monoecious intermediary.

4. Highly organized sex-determining mechanisms of dioecious plants can evolve from single gene segregation, as shown (a) by the synthesis of dioecious strains of hermaphrodite species, and (b) by the analysis of sex in dioecious species of plants.

5. This evolution is illustrated by the study of sex chromosomes and shows the transition from the type where the two chromosomes are indistinguishable to highly complex systems. Lower stages are commonest in plants, higher stages in insects and mammals.

6. Male sterility which has become established in wild populations of hermaphrodite species is cytoplasmically inherited in all known cases. This system is not considered to be a transitional stage in the development of dioecy but rather as another stable outbreeding mechanism.

7. Sporadic distribution and lower differentiation of sex chromosomes agree in showing that sex differentiation is short-lived in flowering plants. This is to be expected since sex separation in sessile organisms, in comparison with other outbreeding systems in plants, suffers from two shortcomings: (a) that the degree of outbreeding is inflexible, and (b) that utilization of gametes is wasteful. Male sterility is not subject to these limitations.

8. This gametic wastage is mitigated by changes in the sex ratio. Such changes are effected by the differential pollen tube growth of male and female determining pollen grains. For this reason it is the male that usually becomes the heterogametic sex.

9. We can thus have an intelligible evolutionary interpretation of sex in plants when we consider (a) the sporadic occurrence of sex separation, (b) the simplicity of the mechanism determining this separation, and (c) the great diversity of other sexual breeding systems.

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EXTEROCEPTIVE FACTORS IN SEXUAL PERIODICITY

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It is well known that the gonads through their respective hormones act upon the accessory reproductive organs and other tissues concerned in the sexual cycle and that the anterior pituitary acts similarly by its hormones upon the gonads. Moreover, it would appear certain that many external factors which regulate the cycle act through the intermediation of the central nervous system upon the anterior pituitary, this gland playing the part of a liaison organ between the nervous system which is affected by stimuli from without and the endocrine system which undergoes more or less rhythmical internal changes associated with the gonadal functions.

In this article it is proposed to present and summarize the evidence recently collected as to the parts played by exteroceptive stimuli which alter or regulate the gonadotrophic activities of the anterior pituitary. But before dealing with the experimental researches bearing upon this subject it will be convenient to refer briefly to the relevant evidence derived from the observational study of the higher animals living under natural conditions or in states of domestication and confinement.

I. OBSERVATIONS ON SEXUAL PERIODICITY IN MAMMALS

It has been observed that whereas there is no month in the year which is not the sexual season of some species of mammals, yet for the species in question this season is most regular. Although there is a natural rhythm in reproductive periodicity depending upon an alternation between comparative rest and activity, this rhythm is brought into relation with seasonable environmental change in such a way that the young are born at a favourable time of year. This coordination is seemingly effected by reactions on the part of the animal to appropriate stimuli,

more particularly in association with the breeding season. It has often been pointed out that most animals breed in the spring and presumably in response to some change or changes which occur at that time. Thus the insectivores, carnivores, rodents and non-ruminating ungulates with very few exceptions breed in the spring in both northern and southern hemispheres. In subtropical areas there may or may not be a breeding season with the mammals inhabiting them and its occurrence is to some extent related to latitude. Some of the rodents in temperate climates are exceptional in having very extended breeding seasons or in breeding almost continuously (Marshall, 1937). With other orders of mammals there would appear to be some variation in the seasonal response of the reproductive organs. With marsupials nearly every month of the year may be the breeding season of some species, but many of them live under comparatively uniform conditions, at any rate in regard to the incidence of daylight (Wood Jones, 1923-5). Sexual periodicity in bats has been discussed and the main facts summarized by Baker & Baker (1936) and Baker & Bird (1936). The fruit bats breed in the autumn (or corresponding time even though the environmental conditions are almost uniform throughout the year as in the New Hebrides) in both hemispheres. The insectivorous bats that hibernate copulate in the autumn and ovulate in the spring, but many copulate again in the spring. *Miniopterus* in the New Hebrides has a sexual season in the southern 'spring'. For the primates the evidence has been summarized by Zuckerman (1932). Although some species are said to have restricted seasons, Zuckerman concludes that, speaking generally, 'monkeys and apes, like man, experience a smooth and uninterrupted sexual and reproductive life'. There is evidence, however, that primitive man had an annual sexual season in spring.

The ruminating ungulates with a few possible exceptions stand out in contrast to most mammals in breeding in the late summer or autumn when the daylight is diminishing and this applies to species both in the northern and in the southern hemispheres. Teleologically this may be regarded as a device to secure that the young shall be born at the most favourable season for their development, that is, in the spring, and, as already said, a similar explanation may be made for the sexual seasons of other animals. This, however, is no solution of the physiological problem as to the causes of seasonal sexual activity in the individual, and these causes differ in passing from group to group or even from species to species or from breed to breed. It has been pointed out that for animals living under tropical or subtropical conditions where the seasons are less marked or non-existent there is often a much less pronounced sexual periodicity, but even in these regions the rhythm of reproduction may be closely related to annual occurrences and it is probable that ecological factors may play a part in determining the time of breeding.

The domestic animals, at least in many instances, have to a considerable extent freed themselves from the influence of seasonal changes and their oestrous cycles are commonly completed in a shorter time. Thus the domestic bitch may breed at any time, though maintaining an internal sexual rhythm as shown by the cycle which is typically one of six months independently of environmental changes. Heape (1900) states that the dogs of Danish Greenland have only one annual

breeding season and resemble the wolf and the jackal in only breeding in the spring. The dingo of Australia also breeds only in the southern spring (Wood Jones, 1923-5). In a similar way the domestic cat may have two or three sexual seasons in the year whereas the wild cat breeds only in the spring and the allied African wild cat in the southern spring (Fitzsimons, 1919-20). The domesticated rabbit when kept warm and well fed may breed all the year round, whereas the wild rabbit usually breeds only from February to May though it may have a second breeding season at harvest time. Similarly in Australia the rabbit breeds in the southern spring but may have a second breeding season or in some parts breed for the greater part of the year.

Species of mammals which have been transferred from the northern hemisphere to the southern commonly reverse the time of their breeding seasons and this is the case with ruminants which in Europe breed in the second half of the year. Thus the sheep and goats, red deer, fallow deer, moose, wapiti, Virginian deer and chamois reverse their breeding seasons. The thar in New Zealand ruts, not at the reverse of the time for this species in India (where it breeds in September and October) but at the reverse of its rutting time in the Zoological Society's Gardens in London (where it bred in November, the season in New Zealand being late April or May) (Donne, 1924; Marshall, 1936, 1937). It would appear, however, that tropical or subtropical deer which in their native countries tend to breed at any time have little or no capacity for responding to any seasonal changes (such changes being for them non-existent in a state of nature) and tend similarly to breed at their ancestral time or at any time in their new environment. This is the case with the axis, Javan rusa, and hog deer and Reeve's muntjac, as well as with the thamin or Eld's deer and the Indian nyulghai antelope. According to Bodenheimer (1938) gazelles of various species in the Zoological Gardens at Cairo retain their normal breeding season, although there is a general uniformity in external conditions.

The evidence presented shows that in all species of mammals there is a sexual rhythm which is presumably dependent upon an endocrine cycle but that this is usually, though not always, adjusted to external seasonal change. That the recurrence of the sexual periods is not due entirely to endocrine factors is shown especially clearly by those individuals which belong to species or breeds that ordinarily have only one sexual season annually, yet can be induced to have two seasons by transferring them across the equator from one hemisphere to the other. Thus it is known that Southdown ewes if sent to Australia or South Africa when in a state of pregnancy may, after lambing, have a second sexual season within the year and subsequently conform to the reversed conditions of the southern hemisphere, but the complete adjustment takes about two years. Similarly, for red deer shipped to New Zealand, the adjustment takes about the same time, the oestrous cycle being at first accelerated. Moreover, with hinds brought from New Zealand to England and arriving in March, the original gonadal rhythm was at first maintained and the hinds came on heat in April at the New Zealand time. Their next sexual season, however, was in December-January and their third sexual season in England

was October, or the normal time for British deer (Marshall, 1937). Further, it is now known that such phenomena are not confined to ungulates and that ferrets behave somewhat similarly, for some of these animals on being sent to South Africa from England bred in October or shortly after their arrival and later changed over permanently to the seasons of the southern hemisphere. On the other hand ferrets sent to Kenya where conditions remain more uniform (at any rate in regard to the length of day) experienced oestrus at the end of the year and some individuals afterwards bred irregularly as though not in response to any recurrent environmental factor. According to Zuckerman (unpublished), the vizcacha, a South American rodent, may breed in any month of the year under conditions of captivity in England, whereas in Argentina it breeds only in autumn (April).

II. OBSERVATIONS ON SEXUAL PERIODICITY IN BIRDS

The subject of seasonal periodicity in birds has been studied at great length by Rowan (1926, 1938¹) whose experimental investigations were the starting-point of much subsequent work. As is well known, most birds breed in the spring when daylight is increasing, yet as Baker (1939) has remarked, the general evidence shows that light can be only one of the factors concerned. It has been suggested that the increase in the size of the gonads and the greater quantity of hormones presumably secreted in correlation with this increase may be the source of the migratory impulse which is associated with the stimulus for breeding. In the case of those species which migrate in the first part of the year from the southern to the northern hemisphere, the gonadal increase is said to commence before the migration starts and in such cases it must be supposed mainly to be the result of a gonadal rhythm. At any rate it is not due to increase in daylight (Rowan & Batrawi, 1939). With non-migratory tropical birds which live where there is little or no variation in daylight seasonal breeding may still occur. In the unvarying tropical climate of the northern New Hebrides the passerine bird *Pachycephala pectoralis* is as seasonal in its reproduction as are the birds of temperate climates (Baker *et al.* 1940) and the environmental change controlling the breeding season is unknown. Moreau (1936) writing of the birds of East Africa concludes that the limitation in the times of breeding in the mountain forest species is 'due to a variety of congruent causes, both limiting factors and stimuli, external and internal'. Murphy (1936) states that in the Atlantic equatorial isles Fernando de Noronha and St Paul Rocks, most of the birds have no breeding season, for eggs and young may be found in any month of the twelve. Penguins which have invaded various warm-temperate and tropical environments from the south may be occupied in breeding for nearly the whole year, and in the Zoological Gardens the black footed species can breed several times in one year. It would appear that these birds have freed themselves from external seasonal influences like the domestic dog among mammals. Speaking generally, however, in the southern hemisphere most native birds breed in the southern spring and this is true also for birds imported from the northern hemisphere (Rowan, 1938). Thus in New Zealand, the starling, linnet, red-poll,

¹ *Biological Reviews*.

sparrow, thrush and hedge-sparrow conform to the sexual cycle in that country (Marshall, 1937; this paper gives references). Of migratory birds which breed in the southern hemisphere the mutton bird of New Zealand and certain other species of shear-water (*Puffinus*) are examples. They breed in the south (South America, Australia and New Zealand) in the second half of the year and migrate across the equator to the north (where they do not breed) in the first part of the year (Murphy, 1936).

Birds which have been transported from the southern to the northern hemisphere and kept in captivity or in a state of domestication or semi-domestication generally adapt themselves to the northern seasons in a short time and this is true not merely of the species but of the individual birds. There are, however, some exceptions, e.g. the hooded parakeet and Brown's parakeet from North Australia, which retain their original breeding season in October (Marshall, 1936, 1937). Witschi (1935) states that tropical weaver finches from Africa retain their original cycles in America. Baker & Ranson (1938) have made an extended study of this question citing all the available records (with full references). These show that whereas most birds adjust their sexual cycle to their new environment, with some the original oestrous rhythm is a still stronger factor since they maintain the cyclical periodicity of their former natural environment. On the other hand, the European white storks kept captive in Peru breed in the southern spring, laying eggs in October and thus reversing their sexual cycle. This observation is of exceptional interest since the stork is by nature a migratory bird which crosses the equator and migrates far south annually but breeds naturally in the northern hemisphere.

The actual occurrence of breeding in birds in any particular country or locality may depend upon ecological factors of which the condition of the nesting site may be of great importance. Thus Lack (1933) found that with the Arctic terns on Bear Island in the absence of a suitable site breeding may be postponed or not take place at all. Lack and also Baker (1938) have given other examples. Lack concludes that with the Arctic terns breeding is regulated by the nervous system.

In a later paper Baker (1939) has reverted to the question as to the relation between latitude and breeding seasons in birds and the following is a summary of his general conclusions: 'As one goes north from the temperate latitudes one finds a general tendency for the egg-laying seasons of birds of all kinds to start later and later at the rate of some 20 or 30 days per 10° of latitude. As one goes south from the temperate latitudes into the northern tropical and equatorial zone, one finds a general tendency for the Accipitres, Coraciiformes and, to a less extent, the Passeres, to start their egg-laying earlier and earlier. The Charadriiformes, Grallae, Herodiones, and Anseres behave differently. In the northern hemisphere they tend to breed later in the tropical and equatorial zones than in the subtropical and temperate. There is a general tendency for birds in the tropics to reach the height of their main breeding seasons somewhat before the sun passes over head. Two breeding seasons in the year are quite common, but birds which breed only once select either the northward (Accipitres, etc.) or southward (Grallae, etc.) swing of the sun.' 'It is thinkable that the stimulus provided by the sun is its light (visible

and ultra-violet) which is at its maximum intensity when it passes overhead' (Baker, 1937). 'The main proximate causes of the breeding seasons of birds in nature are thought to be temperature and length of day in the boreal and temperate zone and rain and/or intensity of insolation near the equator. The time of arrival from migration is often an important factor. Much egg-laying occurs when the days are getting shorter, and indeed it often proceeds rapidly when they are decreasing in length and only between 11 and 12 hours long. There is, however, little egg-laying when the day is shorter than 11 hours, and almost none when it is less than 10' (Baker, 1939).

III. EXPERIMENTS WITH LIGHT AND ULTRA-VIOLET IRRADIATION

It is of course obvious that for breeding to occur successfully in any kind of animal the appropriate environmental conditions must be present. The animals must be provided with suitable food, and a proper degree of warmth, etc., while various other ecological factors which vary in different species may be essential. Most of these factors, in a state of nature, work irregularly and are difficult to determine experimentally, so that comparatively little work has been done on their action. In the case of light, however, we are dealing with a factor which is constant and regular over wide areas and hence it was not unreasonable to suppose *a priori*, that differences in the amount and duration of light are a factor of greater importance and more widespread application in seasonal reproduction (which is also, generally speaking, wonderfully regular) than other factors contributing to sexual periodicity. This conclusion has been fully justified by experimental research.

The first to show definitely that light was a cause of cyclical reproductive activity was Rowan (1926) who conducted an investigation on the migratory junco finch of America. The main conclusions were afterwards extended to mammals (voles and ferrets) by Baker & Ranson (1932) and Bissonette (1932*a*). Since that time a large amount of work on the effects of artificial irradiation has been done on many different sorts of vertebrates, mostly birds and mammals, and this has been summarized in papers by Bissonette (1936, 1937), Marshall (1936) and Rowan (1938). In the present article, therefore, with few exceptions, only the more recent work will be recorded. It should be mentioned, however, that according to Rowan (1938) exercise may be a contributory factor in the causation of gonadal activity, since junco finches and sparrows subjected to increased periods of mechanically induced wakefulness in total darkness for four weeks attained full breeding condition. It seems possible, nevertheless, that such results may be actually brought about by other exteroceptive stimuli of various kinds. In the case of ferrets at least, animals used for 'rabbiting' do not come on heat before the normal season whereas those confined to hutches, where they can take hardly any exercise, experience oestrus in winter when artificially irradiated. That the effective stimuli vary with different kinds of animals is shown by the facts that whereas starlings react most to long-waved red light, being unresponsive to green, blue and ultra-violet stimulation (Bissonette, 1932*b*), ferrets on the other hand are usually

most responsive to ultra-violet rays, though the effects begin with the red and extend throughout the visible part of the spectrum.

A later investigation (Marshall, 1940) showed that with female ferrets subjected to different degrees of intensity of light irradiation, as measured by putting them at different distances from a 1000 W. lamp, the acceleration of the oestrous cycle was roughly correlated with the degree of intensity. Feeding vitamin D to anoestrous ferrets did not result in accelerating the cycle, the animals coming on heat at the normal time, thus indicating that the oestrus in the irradiated animals was not due to the formation of vitamin D.

The paths of transmission of the stimuli in ferrets has formed the subject of an investigation by Le Gros Clark *et al.* (1939) who after confirming the older observation that ferrets did not come on heat or else did so much later than the normal time when the optic nerves had been divided found later that the normal response to visual stimulation can nevertheless occur in the absence of the visual cortex and the superior colliculi. The conclusion is suggested that the visual response depends on impulses passing either to the ventral nucleus of the lateral geniculate body or to the subthalamus by way of the accessory optic tracts.

Ringoen & Kirschbaum (1939) found that with sparrows there was no gonadal response to light if the eyes were covered. Riley & Witschi (1937), however, obtained no response to light on the part of the ovaries in any case.

Benoit (1936-7, 1938) working on the duck found that removal of the eyeball and severance of the optic nerves did not inhibit capacity to breed, and that artificial light directed through a fine glass tube into the eye-socket and on to the pituitary was effective as a stimulus to the reproductive organs.

In recent experiments on the white-footed mouse (*Peromyscus leucopus noveboracensis*) Whitaker (1940) found that animals blinded by removal of the eyes were not rendered sterile but exhibited no cyclic tendency to sexual activity. Those kept in continuous darkness exhibited a lowered and entirely non-cyclical reproductive activity. Furthermore, with light of the low intensity of one foot-candle power breeding took place throughout the short-day portions of the year, the animals failing to go into a state of anoestrus. Even at lower temperatures when the litter could seldom be successfully reared the mice did not go into anoestrus if provided with sufficient additional light.

Bissonette & Cseh (1937) working on the raccoon have succeeded in accelerating the oestrous cycle, obtaining two litters of young in one year, thereby increasing the fecundity. These results have also been obtained by luminous irradiation.

Experiments have also recently been done on the greenfinch by van Oordt & Damsté (1939) who in a general way confirm the original observations of Rowan & Bissonette. Birds placed in the dark at the beginning of May when they were in full song were killed at varying intervals. It was found that in the dark both the testes and the ovaries decreased considerably in size and spermatogenesis came to an end. When birds were brought into the light in August after being in the dark their gonads increased, spermatogenesis restarted and the birds began to sing. Moreover, putting the finches in the dark caused them to moult in June instead of

at their usual time in August, and bringing them into the light brought them into full breeding condition again.

Burgar (1939) found that to effect spermatogenesis in the starling light duration must be for nine hours, and there must be periodic increases (as from 9 to 9 $\frac{3}{4}$ hr.). Ringoen & Kirschbaum (1939) found that with the sparrow precocious development of the gonads by increasing light rations occurred generally in the early spring and autumn but the female did not respond so much as the male.

Hemmingsen & Krarup (1937) have shown that there is a correlation between the oestrous phenomena and the ordinary daylight diurnal rhythm in the rat. Heat and also muscular activity (as recorded by 'activity cages') are at their maximum in the dark. All the phenomena (mating instincts, cyclical changes in the vagina, and the correlated increase in activity) are shifted 12 hr. if an artificial day-night rhythm is established by exposing the animals to light in the night and to darkness in the day. An 8 hr. alternating rhythm of light and darkness, however, was not followed by adjustment. Constant light was found to stimulate vaginal cornification and to induce heat. The general considerations adduced in this article make the presumption probable that Hemmingsen & Krarup produced their results through exteroceptive stimuli acting upon the pituitary and relayed by hormonal action from this organ to the reproductive system. Browman (1936) working contemporaneously on the albino rat obtained similar results to those of Hemmingsen & Krarup, and found that the oestrous rhythm was unaffected by blindness or continuous darkness.

The effect of increased daily illumination and of reversed day and night conditions on the oestrous cycle in the mouse have been investigated by Gresson (1940). Females were kept in darkness for 7-8 hr. during the day and under bright electric illumination for the remainder of the 24 hr. Controls under normal conditions were also kept. It was found that 'long day' conditions accelerated oestrus and induced copulation in mid-winter. Reversed day and night conditions brought about daytime pairing. With the control mice in the spring copulation occurred more readily in the daytime than at other seasons of the year, but among the experimental mice copulation was more frequent than among the control animals.

Among the lower vertebrates breeding outside the ordinary season has been induced by methods of light control in both fish and amphibians. With sticklebacks by gradually increasing increments of artificial light mating and nest-building with normally developing eggs have been brought about in January and February and long before the normal time (Tinbergen, quoted by Rowan, 1938). Hoover & Hubbard (1937) have induced spawning in the rainbow trout in December instead of March. In the brook trout which normally spawns in the autumn, Hoover, by first augmenting the light ration in February and then decreasing it has caused egg production in August. March (1937) has shown that augmented lighting can induce breeding in frogs. Spaul also (quoted by Rowan, 1938) by long continued illumination has caused breeding in frogs, newts and salamanders as well as in minnows during autumn and winter. It is known too that gonadal activity in the

lizard *Anolis* is affected by light and active spermatogenesis occurs in response to stimulation by light (Clausen & Poris, 1937; Evans & Clapp, 1940).

It should be emphasized that increases in light have no effect on the breeding cycles of animals if the pituitaries be removed, and that the same effects as those produced by light can be evoked at any season by the injection of the gonad-stimulating principle of the pituitary. Again, Bissonette (1936) has shown that the pituitaries of stimulated ferrets undergo histological changes similar to those of castrated animals, large clear cells being produced. The effects of stimulation, however, are not permanent, for the animals eventually go into a state of sexual rest in spite of the continuation of the stimulating agents used. The existence of this refractory period and its significance in relation to the sexual cycle have been emphasized by Bissonette (1937). As possibly bearing on the same point, it may be remarked that Riley (1937) found that young sparrows in their first autumn in early October respond more readily to increased lighting than do adult males that have recently completed the active phase of the cycle and since regressed.

It has been stated above that all animals do not respond to an increase of light, exceptions occurring among both mammals and birds. Thus the guinea-pig is little affected by changes in the amount of light (Dempsey *et al.* 1934) and the same is the case with the spermophile (Johnson & Gann, 1933). Guinea-pigs, however, like the deer and other ruminants mentioned above, are tropical animals and so living under comparatively uniform conditions as regards light and temperature probably do not possess the capacity to respond to those seasonal conditions which are the main factors in fixing the periods of breeding among animals living away from the equator.

Among birds also some species do not respond to light increase, e.g. the guinea-fowl (Scott & Payne, 1937) which likewise inhabits the tropics.¹

It has been shown, however, that some animals inhabiting apparently unvarying tropical lands are as seasonable in their reproduction as others living in temperate climates. The bats described by Baker & Baker (1936) and Baker & Bird (1936) are examples from among mammals. Among birds also the garden whistler (*Pachycephala pectoralis*) inhabiting the northern New Hebrides is as seasonal in its reproduction as are the birds of a temperate climate (Baker *et al.* 1940). There is said to be no seasonal change in diet and, as remarked already, the environmental change, if any, controlling the breeding season is unknown.

That there is variation in passing from species to species is shown also by the genus *Pachycephala*, for the egg-laying season for the species in the tropics of Australia is quite different from that of the bird in approximately the same latitude in the New Hebrides. Moreover, among the Antarctic penguins the Adélie penguin breeds in the warmest and lightest time of the year whereas the Emperor penguin lays its eggs in the dark. In some species of mammals the gonads begin their annual development in winter before the days begin to lengthen and in birds which

¹ In the common sparrow, which breeds very freely in the wild state in spring and summer, artificial light stimulation favours gonadal development in the male but not necessarily in the female (Riley, 1937; Ringoen & Kirschbaum, 1939; Riley & Witschi, 1937).

migrate from the southern to the northern hemisphere the periodic gonadal enlargement does not commence until shortly before migration starts, the gonads remaining small during the long period of residence in the south when the daylight is very long (Rowan, 1938). Here the periodic enlargement must be correlated with a reproductive rhythm which nevertheless is brought into relation with seasonal changes by the influence of daylight—more especially just before breeding. There are, however, some birds which breed in the autumn. Thus Rooke (1935) describes the Newfoundland red crossbill and the pine grosbeak as having well-developed gonads in August and probably breeding in September.

The outstanding fact remains, however, that in nearly all animals with a sexual periodicity breeding phenomena occur in response to seasonal change, and in the majority of these (but not in all) as shown by observations under both natural and experimental conditions, the principal stimulus is increase of light. In many of the investigations temperature and food as factors have been generally eliminated by control observations, though in some cases such factors may not merely condition the phenomena but play a part in determining the sexual cycle. There is a well-attested belief that for red deer a fall of temperature is essential to bring the animals into their full autumn rut (Marshall, 1937) but this cause cannot be one of general influence for experimental lowering of the surrounding temperature was not found to have any effect in bringing ewes into a state of oestrus. On the other hand, it is probable that with some species of vertebrates a variation in the environmental temperature is a necessary factor in the cyclic activity of the reproductive organs. This is indicated in the ground squirrel among mammals (Wells & Zalesky, 1940) and in the four-toed salamander among amphibians (Branin, 1935). In the ground squirrel Wells & Zalesky found that by maintaining a constant temperature throughout the year spermatogenesis correlated with large accessory male organs could be made to continue for twelve months. According to Lee (1926) the effect of a low environmental temperature is to lengthen the cycle in laboratory rats. In the toad *Bufo cognatus* Bragg (1940) found that breeding occurred only if the temperature was above 12° C. and then only after rain. Many other amphibians are similar.

Apart from Rowan's observations there is little indication that increased exercise accelerates gonadal activity and with mammals there is no such evidence.

IV. SEXUAL EXTEROCEPTIVE FACTORS CONTROLLING THE CYCLE IN MAMMALS

We may now consider the evidence as to the part played by exteroceptive stimuli arising from the relations between the sexes and between the mother and her offspring in controlling or modifying the phases of the sexual cycle. The best known examples of this kind of phenomenon are probably those supplied by such species as the rabbit, the ferret and, more recently, the ground squirrel (Foster, 1934) which only ovulate in response to the stimulus set up by coition or by the orgasm. In such animals sterile coition is usually followed by pseudo-pregnancy with mammary development and secretion. The stimulus, therefore, causes a switch over from the oestrous or follicular phase to the luteal phase. The switch cannot

be effected in the absence of the pituitary whereas it can be produced by the injection of anterior pituitary or anterior pituitary-like extracts. The presumption is that ovulation in these animals is normally due to a nerve reflex involving the pituitary but that the stimulus may be carried by several nervous paths. It is not necessarily started from the vagina and vulva since local anaesthesia of these parts need not inhibit it after coition. Ovulation can occur also after cervical and thoracic sympathectomy and in the absence of any nerve pathway to the ovaries and after ovarian transplantation to another part of the body (see Marshall, 1936).

Marshall & Verney (1936) found that electrical stimulation of the central nervous system (either brain or lumbo-sacral section of the spinal cord) in the rabbit caused ovulation or the formation of haemorrhagic follicles and in the former case switched the cycle from the oestrous to the luteal stage. Ovulation, however, did not usually supervene until 17-24 hr. after the stimulus instead of the usual 10 hr. Ball & Hartman working also on the rabbit have obtained similar results (Hartman, 1939). Harris (1937) found that local stimulation of the hypothalamus (both in the tuber cinereum and in the posterior hypothalamus) as well as of the pituitary itself caused ovulation in the rabbit or ferret but haemorrhagic follicles were sometimes formed instead of the follicles discharging, in just the same way as often occurs after the injection of anterior pituitary-like principle. Haterius & Derbyshire (1937) also produced ovulation by stimulating an area above and anterior to the optic chiasma. In the rat which normally ovulates spontaneously mechanical stimulation of the cervix uteri prolonged the life of the corpora lutea (just as sterile coition does). (For references see Marshall, 1936.) Further, Harris (1936) showed that electrical stimulation through the brain had the same effect, switching the cycle from the follicular to the luteal stage. Moreover, Selye & McKeown (1934) found that mechanical stimulation of the nipples without withdrawing the milk may prolong the luteal phase in rats and mice. The frequent inhibition of menstruation by suckling in women may possibly be similarly accounted for. In the same way the well-known fact that sucking is normally essential for the continuance of lactation may be accounted for. Furthermore, according to Deschlin (1938) the nervous stimulus resulting from suckling is capable of modifying the structure of the anterior pituitary, since in rats whose ovaries are removed immediately after parturition the castration effects upon the hypophysis (which normally occur after removal) are arrested if the animals are allowed to continue suckling their young.

Some further light has been thrown on the nervous mechanism of ovulation by injection experiments with drugs and other substances. Marshall *et al.* (1939) by injecting picrotoxin, a stimulating drug acting on the nervous system, were able to induce ovulation in the rabbit but the follicles sometimes did not rupture until 48 hr. afterwards, and sometimes enlarged cystic or haemorrhagic follicles were formed without ovulation. Other convulsive drugs such as strychnine, apomorphine and coriomyrtin had no effect. It had already been known that various substances of animal or vegetable origin (yeast, etc.) as well as copper salts, zinc sulphate and iron salts promoted ovulation (Friedman & Friedman, 1924; Maxwell, 1924).

Fevold *et al.* 1936) but it was usually supposed that these had an augmentor effect on the pituitary hormone. Copper salts could cause ovulation when injected by themselves intravenously (Fevold *et al.* 1936) and their action was thought to be catalytic on the synergism between the follicle-stimulating and lutealizing hormones. Emmens (1940) has also found that copper and cadmium salts will cause ovulation in oestrous rabbits, but not salts of barium, cobalt, gold, iron, manganese, nickel, silver or zinc. He suggests that the effect is a stimulating one on the pituitary. The action is a delayed one like that occurring in the other experiments described above. In a recent communication, Brooks *et al.* (1940) have described ovulation in the rabbit following upon the injection of copper acetate, picrotoxin and metrazol, but transection of the hypophysial stalk before or shortly after the injection was found to inhibit the result.

Harris (1937) had previously found that lesions of the stalk of the pituitary caused genital atrophy in both male and female rabbits. Brooks (1938), who confirmed Harris as to failure to ovulate after transection of the stalk, states that the operation did not necessarily inhibit desire or cause sexual atrophy. The ovaries showed no marked retrogressive changes and the follicles could usually be made to discharge after injection of pregnancy urine and serum. The corpora lutea also were normal structurally and functionally and the uterine effects were normal for pseudo-pregnancy (Brooks & Lambert, 1939; Brooks *et al.* 1940). Westman & Jacobsohn (1937) found that if the pituitary stalk be first cauterized ovulation did not occur after stimulation through the brain. If the stalk were cut 2 hr. after stimulation, ovulation could occur followed by short-lived corpora lutea. Animals injected with copper salts up to 5 hr. before stalk transection might ovulate (Brooks, 1940).

Richter (1933) found that transection of the stalk in rats caused prolonged dioestrous or pseudo-pregnancy periods, the cycles extending to 12 or 16 days. The significance of this is obscure, but it would appear to be further evidence that the anterior pituitary is a factor in controlling the cycle.

Uotila (1940) also as a result of stalk transection in rats has shown that environmental changes can modify the normal sexual rhythm by impulses which normally reach the hypophysis through the stalk or to a lesser extent through the cervical sympathetic system.

Collin (1937, 1938) has described the morphology and physiology of the nervous factors in pituitary activity, giving a complete bibliography. He states that there are two affecting centres, one hypothalamic and the other sympathetic. He discusses the exteroceptive reflexes with pituitary relays, including those starting from the retina and from olfactory sensations, as well as interoceptive reflexes with pituitary relay (factors determining milk secretion, etc.). He states that double superior cervical ganglionectomy is followed by profound structural modifications of the pituitary such as (in the rabbit) cyanophilic changes in the anterior lobe and secretion of colloid, the organs then becoming chromophilic and showing other modifications.

Experiments by Bard (1935) and by Bard & Rioch (1937) and by Brooks (1937, 1938) indicated that if there is a controlling 'sex centre' in the nervous system it

(1940), however, could not find any conclusive evidence that penguins are comparatively affected by numbers in their egg-laying operations, for the processes appeared to run approximately at the same time in both the small and the large communities. Roberts states further that for penguins, as for so many other birds, the presence of a satisfactory nesting condition is an essential stimulus for egg-laying. In the cuckoo also Chance (1940) has shown that the bird will not lay if it cannot find a suitable nest.

It is the exception among birds for ovulation to take place spontaneously at the breeding season as it usually does among mammals. Gallinaceous birds, however, do not appear to require any stimulus from the male, and domestic ducks and geese, like poultry, ovulate and lay spontaneously under suitable environmental conditions. It is known also that owls, parrots and some other birds do so occasionally in captivity, but in the case of the parrot preening or stroking the head and neck with the hand may supply a stimulus and cause the bird to lay an egg.

Among certain species of lower vertebrates ovulation and oviposition may depend normally upon the presence of a male just as in some birds and mammals. Hartman (1939) remarks that certain species of lizards seem to require the stimulus of sex play to make them ovulate. With amphibians amplexus seems essential in some frogs and toads, but not in all. Waring *et al.* (1941) express the view that in the anuran *Xenopus* the ovaries are close to the threshold of ovulation at all seasons. In this animal oviposition immediately follows ovulation, but in *Rana* the eggs accumulate in the lower third of the oviduct and are expelled in bulk. This expulsion is probably under nervous control, for it cannot occur in pithed frogs. With urodeles there is an indication that courtship plays a part in the stimulus for the deposition of the spermatophore and its appropriation by the female (Noble, 1934).

Among fish it is obvious that the females, probably in most species, ovulate apart from the male, and Hartman (1939) remarks that viviparous poecilids will lay six to eight batches of eggs after a single mating. The male, however, may initiate the processes. Nevertheless there are some species which normally require the presence of the male in order that the eggs may be released. Thus Hamlyn-Harris (1931) states that the American trout-gudgeon will not ovulate unless the male in nuptial condition engages the female in sex play and kneads her body with his mouth. Again, Hobbs (1937) writing of the quinnat salmon and the brown trout of New Zealand states that whereas the female chooses the sites and makes the redds (or areas excavated by the fish for the deposition of the ova in the stream bed) the ova can only be deposited if the males are in attendance. Similarly in the brown trout Hobbs says that 'the presence of a male is not necessary to stimulate the female into preparing a redd but is necessary to excite oviposition'. It is very probable that other Salmonidae act similarly and it has been suggested that the baggots (or baggits)—that is, mature fish whose discharge of ova is delayed or does not take place and are found months after spawning time with ovaries full of ripe ova—are females which failed to ovulate owing to the absence of the male. In such fish the ova subsequently atrophy in situ and new healthy ova like those in fish ascending the rivers appear in the ovaries (Neill, 1920).

Evidence as to the part played by the nervous system in controlling another phase of the cycle has been obtained experimentally by Noble (1937). This investigator found that lesions in the corpus striatum in chalcid fishes caused an inhibition of brooding behaviour. Lesions in other parts of the brain had no effect on brooding.

It will be seen that the available evidence concerning the factors controlling sexual periodicity in the lower vertebrates is in general agreement with what is known about the mammalian sexual cycle.

VI. THE MEANING OF SEXUAL DISPLAY

In view of such facts as those narrated above it is easy to see that sexual display and courtship phenomena generally probably serve an important function in producing the necessary synchronization of the male and female reproductive functions without which procreation cannot be successfully accomplished. Such a view was put forward by Eliot Howard (1929, 1935) as a result of his intensive studies in watching birds in a state of nature. The physiological implications of this theory and the experimental evidence in support of it as derived from the study of various species of vertebrates, were summarized in an article on 'Sexual behaviour in birds' (Marshall, 1929) and in the Croonian lecture (1936). It will be apparent that the general conclusions reached have now been reinforced by further evidence, both observational and experimental. They may be stated as follows:

Since the gonad-stimulating hormone of the pituitary will cause ovarian development and ovulation in birds, as in other animals, and since sexual posturing and even the mere association of two individuals will initiate nest-building and cause ovulation, there is a presumption that sexual posturing produces exteroceptive stimuli which act upon the pituitary through the hypothalamus, and so effects the necessary synchronization between the sexual processes of the male and female birds. Herein in all probability lies the biological or race-survival value of sexual display and of the adornment which in many species is taken advantage of to render the display the more effective. The acquirement and development of the aesthetic sense may in a similar way be partly accounted for, that is to say, the possession of such a sense may be biologically advantageous as favouring pituitary stimulation. This hypothesis as to the meaning and value of sexual display avoids what is an insuperable difficulty in accepting the Darwinian theory of sexual selection—namely, that the display, and in fact courtship phenomena generally, usually occur after and not before the period when the pairs of birds are mated. This is abundantly clear to those who have made observational studies of the phenomena. Some of these studies have been briefly referred to in the Croonian lecture (Marshall, 1936), and the main conclusions reached are fully confirmed by such recent work as the extended observations of Brian Roberts (1940) on the penguins and Perry (1940) on the razorbills, guillemots, kittiwakes and puffins. Hartley's paper (1941) on courtship in the swallow suggesting that two birds sitting together stimulate one another may also be mentioned.

There is now indeed a very considerable literature on sexual display among

birds as well as other animals, and it may be said with confidence that apart from 'threat display' and some other forms which have no apparent relation to reproductive phenomena, the performances serve the function of promoting pituitary stimulation, thereby providing the requisite physiological conditions for successful copulation. Beside the publications referred to much additional information on this subject has been obtained, and valuable summaries of the evidence relating to it are given in the books by Fisher (1939) and Stonor (1940) who describe the displays of a great number of birds. Stonor has compared the displays of two quite unrelated groups of birds, namely the paradise birds and the gallinaceous birds, and has given accounts of the probable evolution of the different kinds of activities. He shows that, whereas in birds of paradise variations in form and in display have gone hand in hand, in gallinaceous birds variation in form has largely outstripped the development of display. He stresses one outstanding fact, namely that 'no matter where the special adornments of a bird may be situated, no matter what form they take, they are always combined and synchronized with one another to produce the maximum possible effect'. (For display in British birds see Jordain & Tucker (1938-41).)

Stimulatory display is not necessarily confined to one sex but is often mutual and very remarkable instances are described by Fisher and Stonor. These instances are drawn from many different orders of birds and include guillemots, Louisiana herons, bateleur eagles, gannets, fulmars, albatrosses and grebes. Moreover, there are certain species such as the button quail, the tinamous and the New Zealand paradise duck, where the female is the active partner in courtship, and in some of these the incubation and care of the young are left to the male. Fisher points out further that in the case of communal displays, apart from the advertisement value of the gatherings, the presence of a large number of birds which perform in common helps to key up and stimulate each individual male and female which visits the group. This is in accordance with the observations of Fraser Darling (1938) referred to above. Perry (1938) has obtained confirmatory evidence from studying colonies of roseate terns, and Lack (1939) in describing the communal display of the blackcock expresses the opinion that courtship and copulation may be more efficient at the larger than at the smaller 'leks' or breeding grounds.

With newts courtship is shown in the active movement of the males in front of the females which they often scrape up against, in the meantime vibrating their tails. This takes place *after* the males have dropped their spermatophores which the females are apparently stimulated to pick up, for if the males do not perform the females do not pick up the sperm packets. As Huxley (1941) has remarked there can hardly be any possibility of Darwinian sexual selection since the female cannot know that a particular packet has been dropped by a particular male.

As has been shown above, there is clear evidence that courtship behaviour and even the mere presence of the male may aid in effecting synchronization of the sexual stages of fish. Courtship of the female may consist merely of the male swimming around in the vicinity of the female, or it may take the form of a more elaborate display comparable to the nuptial antics of some birds. It usually lasts

for only one season (Norman, 1936). Norman describes the fighting fish of Siam (*Betta*) as extending its fins to the uttermost and displaying its bright red gills and iridescent colours and quivering with intense excitement. Innumerable examples of the same kind of phenomena might be cited of other species of fish. Among marine fish, where the number of eggs spawned is usually very large, courtship is rare. On the other hand, in those fish which experience courtship and pairing at the breeding season the number of eggs is small or moderate (Norman, 1936). This is clearly suggestive of the importance of functional synchronization of the generative processes in facilitating fertilization in such cases. Noble (1938,¹ 1939) refers to the parts played by courtship and display in favouring such a synchronization, but he remarks that there are other factors to consider. 'It is the nest-building habit of birds and the need for the formation of bonds in species which rely chiefly on visual and auditory cues that have been responsible for the elaborate display of birds. Where the courtship is short but brilliant, the display may be a threat essential to induce female posturing but not producing a marriage bond. Where the courtship is long and with many symbolic components bonds are formed which will hold the pair together for life' (Noble, 1939).

The precise nature of the stimuli which produce the cumulative effect of display must be very variable, and it may well be that a condition such as that implied in the 'Gestalt' conception of Köhler (1930) plays a part in transmitting the necessary impulses to the pituitary and thence to the gonads. According to such a conception response does not succeed stimulus after the automatic manner of a reflex, but the results of a succession of stimuli become organized in mass in the central nervous system, and the response to the regions and parts eventually affected is a consequence of the organized whole.

In view of the considerations here summarized the theory of sexual selection in the form put forward by Darwin must be rejected, while the evidence suggests very strongly that the function of sexual posturing and the display of the adornment which is associated with it is to promote a physiological correlation between the male and the female generative processes, the pituitary body playing an important part in its effective accomplishment.

VII. SUMMARY

1. In all mammals and probably in all vertebrates there is an internal endocrine sexual rhythm with alternating periods of rest and activity. This cycle is usually adjusted to external seasonal changes and with some marked exceptions spring is the period of greatest sexual activity, but there is much specific variation and among ruminating mammals breeding is generally in autumn.

2. Among tropical and subtropical animals breeding is not nearly so restricted to definite seasons, and there is evidence that ecological factors play a part in its recurrence.

3. In individual animals which are made to cross the equator there may be two seasons of sexual activity in the year and the times of breeding are eventually

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versed though the original endocrine rhythm may be partly maintained for some time.

4. Experiments with light and ultra-violet irradiations indicate that with the majority of vertebrates exteroceptive stimuli dependent upon these processes are of primary importance in adjusting the cycle to changing periodic environmental conditions.

5. The evidence shows that the factors in question act through the intermediation of the central nervous system and the anterior pituitary body.

6. The cycle is liable to considerable modification by exteroceptive stimuli arising from the relations between the sexes and between the mother and her offspring. Thus ovulation in various species of vertebrates may depend upon stimuli normally arising from the male but which may be imitated by experimental methods.

7. Sexual display and courtship phenomena among various classes of animals, while undoubtedly in some species serving to keep the pairs together and thus securing a 'marriage bond', have the further and much more general function of promoting an effective synchronization of the male and female sexual processes, thus favouring successful procreation.

8. The synchronization is mainly effected by pituitary stimulation which is often mutual, the stimuli acting through the intermediation of the hypothalamus, as in the case of other stimuli.

9. Whatever the exteroceptive stimulus the anterior pituitary is the regulator of the gonadal function.

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ADDENDUM

1 November

Bissonette (1941), in a paper on the experimental modification of breeding cycles in the goat, has shown that shorter days tend to induce such cycles and longer ones to inhibit them.

Bullough (1939, 1941), working on the minnow (*Phoxinus laevis*), found that experimental darkness caused delay in ovarian development and slight retardation in testicular development. He concludes that the breeding cycle, although governed by an internal rhythm, is regulated by external factors, especially light.

Zalesky & Wells (1940) in a further communication confirm the conclusion that the ground squirrels (*Citellus tridecemlineatus*) require a low temperature ($+4^{\circ}\text{C.}$) to enable them to breed. Control animals showed the usual sexual regression during the summer, the reproductive organs becoming nearly infantile. In the experimental animals the adrenals showed a highly developed cortex as in normal squirrels at the breeding season.

Brookhurst & Dey (1941) have shown that experimental lesions in the hypothalamus of male guinea-pigs abolish a much-reduced sexual activity but do not interfere with the main gonadotrophic functions of the hypophysis, since spermatogenesis and the condition of the seminal vesicles remain normal.

Harris (1941) has shown that copper acetate injected into the third ventricle of oestrous rabbits causes ovulation. He suggests that copper acetate, like picrotoxin, metrolol and cadmium salts, causes ovulation by stimulation of the nervous pathway to the pituitary.

Armstrong has contributed a volume (now in the Press) giving detailed accounts of avian display and its significance. The importance of display in relation to reproduction is fully discussed.

The facts relating to the incidence of the breeding season in deer and other animals, briefly referred to above, are now being separately published (Bedford, Duke of & Marshall, 1942).

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HORMONES IN CRUSTACEA

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I. INTRODUCTION

WITHIN the past decade a rapidly growing interest in the field of invertebrate endocrinology has become apparent. This is evident from the number of reviews of this subject in recent years (Koller, 1929,¹ 1938; Hanström, 1937^b, 1939; de Lerma, 1936; von der Wense, 1938). To add merely another summarizing paper to this list would be superfluous. Instead, it is my intention to review here the recent reports of hormonal processes in the one group of invertebrates, the Crustacea, in which enough experimental work has been done to warrant critical discussion.

Inasmuch as the prevailing hormone concept is one that has been developed by investigators of vertebrate physiology, experimental evidence for hormonal processes among invertebrates should be judged by the same standards as those used for vertebrate endocrinology. These standards are now almost classical. An endo-

¹ *Biological Reviews*.

crine effect is conclusively demonstrated when removal of a suspected organ leads to the display of characteristic symptoms and when injections of extracts or implantations of the suspected organ relieve the symptoms of the deficiency and restore the normal condition. To this end the present review will cover only those papers which treat of the normal physiology of crustaceans. The less obviously significant papers dealing with effects of vertebrate hormones on invertebrates, or the extraction and effects of invertebrate hormones on vertebrates will be omitted.

Brief surveys of the field will be given at the beginning of each section on the various hormonal processes in crustaceans.

II. THE CHROMATOPHOROTROPIC HORMONE

(1). *Colour changes in crustaceans*

Colour changes in the lower vertebrates have been known for a long time, having attracted the attention of naturalists since the time of Aristotle. Metachrosis in the crustaceans was rather less commonly known, and appears to have been first recorded by Kröyer (1842) in the prawn, *Hippolyte*. The physiological basis underlying this activity was first studied by Pouchet (1872-6) who had been successful in investigating the process of metachrosis in teleost fishes, and was placed on a firm experimental basis by Keeble & Gamble (1900-5).

Colour changes, in crustaceans as well as in other animals, may be of two sorts, morphological and physiological, or in Sumner's (1940)¹ synonymy, quantitative and transitory. The former involve alterations by the deposition or destruction of the integumentary pigment and occur comparatively slowly. Physiological or transitory colour changes, on the other hand, involve the activity of specialized cells containing pigment, the chromatophores, in which the pigment granules may be dispersed through interlacing cell processes so that the animal is dark or coloured; or the granules may be concentrated approximately in the centre of the cell body to form a small punctate mass, resulting in a lightened appearance of the animal. This paper is concerned with the latter division of metachrosis.

The chromatophores, or effectors involved in physiological colour changes, are found directly underneath or within the hypodermis and also in the deeper lying organs of the body through which they may, in many cases, be easily seen because of the transparency of the overlying tissues.

Chromatophores in crustaceans may be classified according to several characteristic features. One method is based on the colours of the pigments contained within the cells; thus, melanophores, erythrophores, guanophores and xanthophores refer to cells containing black, red, white and yellow substances, although there is little information as to the true chemical nature of many of these pigments. A second, histological distinction is made between chromatophores which are single cells and those which are groups of anastomosing cells or a syncytium. A third classification is made according to the numbers of pigments contained within the chromatophores, those containing only one pigment being known as monochromatic

¹ *Biological Reviews*.

while the polychromatic type contains more than one pigment. In the latter class a single chromatophore may possess, as Koller (1927) has pointed out for *Crangon*, four different pigments: brown, white, yellow and red. Scattered among the polychromatic effectors of this animal are also many monochromatic chromatophores. In crabs like *Portunus ordwayi* (Abramowitz, 1935) only monochromatic colour cells occur, containing either white, black, yellow or red pigment. This polychromatism or combination of poly- and monochromatic effectors is in contrast with the physiological monochromatism of the isopod, *Ligia* (Kleinholz, 1937a; Smith, 1938; Sawaya, 1939), in which black monochromatic pigment cells are the effectors chiefly responsible for changes in tint. Crustaceans like *Ligia* necessarily have only a limited range of colour change, but the polychromatic animals like *Crangon* and *Palaemonetes* can alter their tints to match backgrounds of several different colours because the pigments are able to respond in various combinations (Koller, 1927; Brown, 1935a).

The responses of the various chromatophores to backgrounds and to light intensity take place by a movement of the pigment granules contained within those cells. Recent investigators consider the chromatophores and their processes to be fixed in position among the tissue spaces, the migration of the pigment being effected by centrifugal and centripetal streaming of the cytoplasm through these processes. Colour changes are now usually described as concentrations and dispersions of the pigment granules within the chromatophores, rather than as contractions and relaxations of muscle-like cells.

Chromatophores that undergo physiological changes are found among the larger forms of crustaceans, the Malacostraca. In some of these, such as *Uca* (Megušar, 1912; Carlson, 1936; Abramowitz, 1937a, b), the chromatic activity consists of periodic diurnal and nocturnal changes of the melanophore pigment, independent of light and the colour of the background. In others, like *Palaemonetes* (Brown, 1935a, b) and *Crangon* (Koller, 1925-30), there is true colour change, consisting of adaptation of body colour to the colour of the background by the concentration of pigment in some chromatophores and by pigment dispersal in others. To a third type belong crustaceans like *Ligia* (Kleinholz, 1937a; Smith, 1938; Sawaya, 1939), which can change their shade in response to illuminated black or white environments, but which cannot adapt their own colour to match those of various backgrounds. In many decapod crustaceans, colour changes have been observed not to occur, but this may be due to the fact that the integumentary chromatophores are obscured by an opaque exoskeleton. Carlson (cited by Hanström, 1939, p. 104) made windows in the skeleton of some of the large decapods and could follow the pigment concentration and dispersion of the integumentary chromatophores.

In many investigations of physiological colour changes the most obvious component of the chromatic system of the experimental animal is the one usually studied. In the prawns this is commonly the red pigment, while in the brachyurans it is the black. There are scattered observations on the other chromatic components, but those chromatophores which play only a limited part in the process of metachrosis have been relatively overlooked. Knowles (1939) studied the behaviour

and physiology of the guanophores in several Mediterranean prawns, while Sawaya (1939) has recently recorded the behaviour of the xanthophores in *Ligia*.

(2) *Mechanism of colour change in crustaceans*

The first attempts at experimental analysis of the physiological mechanisms underlying colour changes were performed by Pouchet (1872-6), who applied to crustaceans the same methods he had used in studying metachrosis in teleost fishes. He found by removal of one eye of a prawn that the remaining eye was sufficient for maintaining the normal colour changes, but if both eyes were removed, the animal became dark and lost its ability to change colour in response to different backgrounds. Pouchet and many later workers agreed in assigning to the eyes an important part in the regulation of metachrosis. Pouchet undertook to demonstrate nervous control of chromatophores in crustaceans by cutting the ventral nerve cord, but these experiments were without effect; metachrosis occurred apparently in normal fashion both in the innervated and in the denervated regions of the body. Following the early studies of Pouchet (1876) on the colour changes of decapod crustaceans, Matzdorff (1883) published a report on the coloration of the isopod, *Idotea*. He, too, was of the opinion that chromatophoral activity was under nervous control, but decided that the inconclusive results obtained on severing the ventral nerve cord were to be attributed to injury caused by the operative manipulation. Menke (1911), in a study of the rhythmic activity of colour changes in isopods, believed that the mechanism for such responses was based upon physiologically innervated chromatophores, referring in support of this to a figure of an innervated colour cell from the integument of *Philoscia* in Weber's (1881) paper. The implication Menke drew from the illustration was that chromatophoral activity depended upon a local receptor-effector mechanism, a condition which subsequent study of blinded animals failed to confirm (Tait, 1910). Menke also found that section of the ventral nerve cord had no effect on metachrosis in the denervated region.

Despite the absence of effects on metachrosis from experiments involving the cutting of nerves, many later investigators of colour changes in crustaceans accepted Pouchet's conclusions that these effectors were under nervous control (Keeble & Gamble, 1900-5; Fröhlich, 1910; Menke, 1911; Degner, 1912; Piéron, 1914) largely on the basis of the commonly observed result that removal of the eye-stalks caused a total loss of the ability of such animals to adapt themselves to background changes.

In more recent times experiments to test the role of the nervous system in the regulation of crustacean metachrosis were undertaken by Koller (1925, 1927) for the shrimp, *Crangon vulgaris*, and by Perkins (1928) for the prawn, *Palaemonetes vulgaris*. Koller found much the same results reported by the earlier investigators on sectioning the ventral nerve cord. Perkins made various cuts across the abdomen in different individuals, yet none of these cuts prevented normal colour changes in the distal region except those incisions which severed the dorsal blood vessel. When this vessel was cut, the chromatophores of the distal region supplied by the severed portion dispersed their pigment. Similarly, when circulation in a branch of the

dorsal vessel was interrupted, the chromatophore pigment of the region supplied by the branch became dispersed while the rest of the abdominal region continued its normal colour changes. These results suggested the possibility that chromatophoral nerves accompanied the blood vessels, but histological study showed no nerves on these vessels.

Koller (1925, 1927) had also shown that nerves played no direct role in the chromatophoral changes of *Crangon*. The evidence of both these investigators pointed clearly to some blood-borne agent as the controlling factor in crustacean metachrosis. Koller (1927) removed blood from individuals adapted to a black background and injected it into shrimp which had been adapted to a white background, obtaining typical darkening of the recipients through dispersion of the pigment granules within the melanophores. When the converse experiment was tried, that of injecting blood from light individuals into dark individuals, no changes were observed.

Perkins (1928), having abandoned the idea that nerves were directly involved in chromatophoral changes, attempted to locate in *Palaemonetes* a source of chromatophore-activating material that was transported through the blood. After several unsuccessful attempts he found that when several eye-stalks of this animal were triturated in sea water and injected into stalkless individuals in which the chromatophore pigment was dispersed, such injected animals blanched. No effect could be brought about by similar injections into pale *Palaemonetes*; control injections of sea water alone into both pale and dark animals were also without effect. From these observations Perkins concluded that the retina of *Palaemonetes* when stimulated by light from a white background caused the release into the blood stream of a substance from the eye-stalk that concentrated the chromatophoral pigment and thereby produced the pale phase of the colour state. He was unable to find a substance that would disperse the chromatophoral pigment in *Palaemonetes*, and concluded that this phase of the colour change was an inherent property of the chromatophore itself.

These results of Perkins were soon confirmed by Koller (1928), working on *Crangon* and *Leander*. Koller reported further that he had located in the rostral region of *Crangon* an organ whose secretion was responsible for the dispersion of the black integumentary pigment of this shrimp; the dispersing hormone he named 'expantin' and the pigment-concentrating hormone of Perkins he named 'contractin'. Numerous later investigators confirmed the presence of a chromatophore concentrating principle in the eye-stalks of decapod crustaceans (Kropp & Perkins, 1933; Hosoi, 1934; Brown, 1935 *a, b*; Carlson, 1936; Hanström, 1937 *a*; Abramowitz, 1937 *a, b*; Kleinholz & Welsh, 1937) and in head extracts of isopods (Kleinholz, 1937 *a*; Sawaya, 1939; Ståhl, 1938) and demonstrated that the hormone was inter-specific in its effect, extract prepared from one species blanching a dark prawn of another species.

Confirmation of Koller's 'expantin' effect was less readily available. Various American investigators (Perkins & Snook, 1931; Kropp & Perkins, 1933; Brown, 1935 *b*) were unable to confirm the presence of a chromatophore-dispersing hormone

in the crustaceans they studied. Beauvallet & Veil (1934) reported a slight darkening in some cases when *Palaemon squilla* were injected with large doses of extract prepared from the rostral region. Kleinholz (1938) undertook a reinvestigation of *Crangon vulgaris* to test for the melanophore-dispersing hormone. He found that the darkening effected by blood transfer was not a specific hormonal effect. Carlson (cited by Hanström, 1939, p. 97) failed to observe darkening in white-adapted *Crangon* after injection of blood from dark individuals. The results of blood transfer reported by Kleinholz and by Carlson are thus seen to differ greatly from those reported by Koller. The latter author found that blood from dark individuals when injected into white-adapted *Crangon* induced melanophore dispersion in 95 % of the cases, while blood from white-adapted animals, injected in control experiments, had almost no effect (1927, pp. 237-9). Kleinholz injected aqueous extracts prepared from the rostral region of *Crangon* into white-adapted shrimp and obtained darkening in slightly over 50 % of the injected animals. Koller (1928) attempted local destruction of the regions suspected of this endocrine function. He reported that superficial cautery of a delimited area in the rostral region resulted in a permanent loss of the individual's ability to adapt to black backgrounds. Kleinholz, on the other hand, was unable to obtain convincing duplicates of these results; of sixty-nine shrimp on which cautery was done, only nine became permanently pale, the melanophores being maximally concentrated regardless of the fact that the animals were maintained on an illuminated black background. In each of these nine individuals, however, swimming and equilibratory movements were abnormal, leading Kleinholz to the opinion that deep cautery had injured the central nervous system and possibly interfered with the regulation of the secretory tissue responsible for the melanophore-concentrating hormone.

Brown & Ederstrom (1940) prepared aqueous extracts of the circumesophageal nerve commissures of an American species of *Crago*¹ that upon injection into eye-stalkless individuals caused a darkening of the telson and uropods only. The significance of these results is at present doubtful; the response is obviously not comparable to the generalized darkening observed by Koller. The situation at present is such that much more critical evidence will be needed before the existence of a melanophore-dispersing hormone, originating in the rostral region of crustaceans can be unqualifiedly accepted.

(3) *Localization of the origin of the hormone*

After Perkins's report (1928) on the presence of a chromatophore-concentrating substance in the eye-stalk of *Palaemonetes*, there were only a few attempts at discovering the organ or tissue within the eye-stalk responsible for the secretion of the pigmentary hormone. Koller (1930) found that the retinal portion of the eye-stalk of *Crangon vulgaris* had no chromatophorotropic effect, but that the source of this hormone could be more accurately located near the basement membrane of the eye where he found a group of cells which he called the blood gland. Results from

¹ The name *Crago* is sometimes used by American zoologists for the genus which in Europe is more commonly called *Crangon*. The latter name is then transferred to the genus better known as *Alpheus*.

experiments involving cauterization of this organ led Koller to believe that it was the source of the chromatophorotropic hormone. Hosoi (1934) also reported that the largest amount of the eye-stalk hormone came from the middle region of the stalk in *Penaeus japonicus*, and confirmed an earlier discovery by Brown (1933) that traces of the chromatophore-concentrating hormone could be found in the ventral nerve cord. Hanström (1937a) did not consider Koller's organ responsible for the secretion of the hormone, since it had no definite innervation and was absent from stalks of *Palaemonetes vulgaris*.

In a series of studies on the nervous system of Crustacea, Hanström (1931-4) had described two structures in the crustacean eye-stalk which he thought might be secretory in function; these he named the X-organ and the blood gland (the latter was subsequently renamed the sinus gland). Sjögren (1934) investigated the structure of the sinus gland in a number of crustaceans, while Hanström (1937a), Carlson (1935, 1936) and Brown (1940) conducted localization experiments to demonstrate the connexion between the sinus gland and chromatophore-concentrating activity. By cutting the eye-stalks of various crustaceans into portions that contained the sinus gland or the X-organ and testing the efficacy of extracts from these regions in concentrating the chromatophoral pigments of stalkless *Palaemonetes*, Hanström decided that the sinus gland was probably the source of the active hormone. But since he was unable to make a sharp separation between segments containing the sinus gland and those containing the X-organ, this decision could not be conclusive. Carlson (1935) was more fortunate in using the long slender stalk of the fiddler crab, *Uca pugilator*, for his localization experiments. According to this investigator, the middle third of the *Uca* eye-stalk contains the active chromatophorotropic principle, the proximal and the distal thirds being relatively ineffective; histological examination showed that this middle region contained the sinus gland whereas the X-organ was either very small or absent. More definite correlation of chromatic function with these two organs was shown by further work of Hanström (1937a) on the crustaceans, *Gebia affinis* and *Hippa talpoida*. Stalks from these two animals were entirely without effect on the chromatophores of stalkless *Palaemonetes*; histological study showed that both of these organs were absent from the stalk, and when it was found that the chromatophorotropic activity was present in extracts prepared from the heads of these animals, examination revealed the two organs on the dorsal surface of the brain.

This latter situation is similar to the one reported by Kleinholz (1937a) for the isopod, *Ligia baudiniana*. In this crustacean the eyes are sessile, but extracts of the heads blanch dark individuals and had no effect on pale individuals. These results were confirmed by Sawaya (1939) on a Brazilian species of the same genus. Sawaya found, in addition, that extract prepared from the eye-stalks of a xanthid crab, *Eriphia gonagra*, similarly concentrated the melanophore pigment of dark *Ligia*. He located in the eye-stalk of this crab a glandular organ which he thought was the X-organ of Hanström. A sinus gland was apparently not found or described by Sawaya. Ståhl (1938), on the other hand, obtained some rather disconcerting results from histological and injection studies with several crustaceans. Injection of

extracts of the heads of *Diastylis*, a Cumacean, into stalkless *Leander adspersus*, resulted in a characteristic blanching in colour; histological examination of the head of *Diastylis* revealed no sinus gland but an apparently typical X-organ. Injection of head extracts from three isopods, *Oniscus*, *Porcellio*, and *Idothea* into appropriate specimens of *Leander* evoked either a slight darkening of the test animal, or no clear-cut response at all, yet a sinus gland was found in the head of *Oniscus*. The evidence from these experiments of Ståhl is confusing and calls for more detailed study. In the present state they indicate, as did the localization experiments of Hanström and of Carlson, that either the sinus gland or the X-organ, or both, may be concerned in the regulation of metachrosis.

Brown (1940) has attempted to localize the source of chromatophorotropic hormone more specifically in the sinus gland. He measured the magnitude and duration of the chromatophoric effect in both stalkless *Uca* and *Palaemonetes* after injection of extracts prepared from whole eye-stalks of seven different species of crustaceans and then compared the results obtained by injection of extracts prepared from the sinus glands alone of these seven species. His conclusions are quoted directly: 'its effects (injection of sinus gland extracts) have been shown to be 80% or more of that of the whole stalk, and there is no justification for belief that the remaining 20% of the activity originates from any other tissue but rather results from normal and artificially induced escape of material from the gland proper.... As far as the *Uca* black chromatophores and the *Palaemonetes* red ones are concerned, this gland (the sinus gland) will account wholly for the activity of the eyestalk extracts.' In five cases, namely, with extracts prepared from the whole stalks of *Carcinus*, *Libinia*, *Callinectes*, *Pagurus* and *Crango*, Brown failed to measure the total chromatophorotropic effect, so that his figures for the percentage of the total activity contained in the sinus glands of these species are questionable. In the case of *Uca* and of *Palaemonetes* the total effect of whole stalk extracts was measured; in these two species (*Uca* serving as the test animal) the sinus gland of the fiddler crab contained 78% and the sinus gland of *Palaemonetes* contained all of the activity shown by extracts prepared from corresponding whole eye-stalks. Those who have studied metachrosis in *Palaemonetes vulgaris* have recognized it as an animal whose integumentary erythrophores are not wholly reliable for quantitative studies of this function because of their very great variability even under apparently constant experimental conditions. In his use of *Palaemonetes* as a test animal, Brown has given no quantitative indication of the variance among his measurements.

Abramowitz (1937a), who devised the method of standardization for crustacean chromatophorotropic hormone, using stalkless *Uca* as the test animal, has shown that the relation between complete response of the melanophores of the test *Uca* and the concentration of hormone is most sensitive over the range from 0.06 to 1.0 eye-stalks per c.c.; over this range there is a linear relation between the duration of the melanophore response and the concentration of injected hormone. It is least sensitive over the range of concentrations above 1.0 eye-stalks per c.c. The apparent decreased sensitivity over the higher concentration ranges may be due to excretion or destruction of the excessive hormone by the test animals. In all except one of

Brown's (1940) tests, concentrations of four stalks or sinus glands per c.c. were used for injection. The question that arises, aside from the fact that these tests were based on the least sensitive portion of the standard curve, is whether the injected hormone would remain in the bodies of the test animals long enough to give the total possible chromatophoretropic effect or whether the injected extract would be partly excreted or destroyed so that total effect of such high concentrations could never really be measured. Such difficulties could have been obviated had less concentrated extracts been used.

The problem of localization of the chromatic hormones in the crustacean eye-stalk is thus not settled. More detailed knowledge of the roles played by the sinus gland and the X-organ must await results from surgical removal of these tissues. Brown (1940) indicates that he has performed such experiments, but the results have not yet been published.

(4) *Diurnal rhythms and the physiology of metachrosis*

An interesting aspect of metachrosis in crustaceans is the phenomenon of diurnal rhythms in pigmentary activity which, in some cases, may even persist under constant environmental conditions. It is now known that, aside from such animals as *Uca* (Megušar, 1912; Carlson, 1936; Abramowitz, 1937 *a, b*) and *Leander squilla* (Hanström, 1937 *a*) in which metachrosis is independent of the colour of the background, the dominant chromatophoral component of the pigmentary system (usually the erythrophores or melanophores in many crustaceans) is concentrated on an illuminated white background, and is dispersed on an illuminated black background. In total darkness, however, the behaviour of the chief pigmentary component is quite different, the animal blanches owing to a concentration of the red or black pigment granules within the chromatophores. This has been more recently confirmed on *Palaemonetes* by Perkins (1928), Hanström (1937 *a*), Brown (1933), on *Eupagurus* and *Leander* by Stephenson (1932, 1934) and on *Hippolyte* by Kleinholz & Welsh (1937).

The behaviour of this second group of crustaceans in light and in darkness is also repeated under the natural conditions of day and night, so that there is a periodicity in metachrosis. In certain crustaceans, however, this periodicity has been found to persist when the animals are kept in constant darkness, indicating the presence and continued functioning of some internal regulatory mechanism. Gamble & Keeble (1900) were the first to report that *Hippolyte varians*, when kept in continued darkness, became pale at night and remained dark during the day. Kleinholz & Welsh (1937) were later unable to confirm these observations, reporting that the integumentary chromatophores of this prawn could respond directly to light and to darkness, and that the observations of Gamble & Keeble were probably due to absence of constant light conditions in their experiments.

Menke (1911), who studied the chromatic activity of the isopod *Idothea*, observed a periodic colour change that persisted in constant darkness, the chromatophoral pigment being concentrated at night and dispersed during the day. Menke concluded that the persisting rhythm was associated with an internal metabolic

periodicity. Piéron (1914), who believed pigmentary changes in *Idothea* were under direct nervous control, suggested that 'the nervous centres can periodically control the reflex without being directly stimulated by sensory impressions (received by the eyes)'. Kleinholtz (1937*a*) observed a persistence of pigmentary rhythm in the isopod *Ligia baudiniana*. Kleitman (1940) confirmed this observation in the same species. To discern whether the activity was due to a rhythmic cycle of exhaustion and elaboration of secretory material in the sinus gland, Kleinholtz measured the efficacy of extracts prepared from the heads of *Ligia* in the two phases of the pigmentary cycle, but found that both types of extracts were practically equally effective in their chromatophorotropic activity. These experiments indicate that the seat of the cyclic activity is probably in the nerve centres, and that periodic activity of these centres causes rhythmic release and retention of chromatophorotropic hormone from the sinus gland.

The diurnal rhythm of *Uca* (*Gelasimus*) was first described by Megušar (1912) and confirmed by Carlson (1936) and Abramowitz (1937*a*). These crabs are black by day and pale at night, regardless of background and light intensity; the same cycle persists in animals that are maintained in constant darkness. Although macruran crustaceans become dark after eye-stalk removal and consequent loss of the sinus glands, Megušar (1912), Carlson (1936), and Abramowitz (1937*a, b*) have demonstrated that brachyuran crustaceans are different in their colour responses to eye-stalk removal, becoming permanently pale and diurnal periodicity ceasing altogether.

Establishment of a uniform explanation of the physiology of the sinus gland and its relation to the persistent cyclic pigmentary activity does not seem possible from the evidence presented above. Using a standardized method for quantitative assay of crustacean chromatophorotropic hormone in terms of *Uca* units, Abramowitz (1937*a*) found that the hormone contents of eye-stalks of *Palaemonetes* kept on illuminated black, white, yellow and blue backgrounds were practically identical, whereas the hormone content of stalks from *Palaemonetes* kept in darkness was half that in illumination. The two significant features of these observations are, first, the similar amounts of hormone both in the stalks of animals showing continued release of the pigmentary hormone (those on an illuminated white background) and in those of animals showing a subminimal or no release of the hormone (individuals on an illuminated black background) and, second, that amount of hormone in the stalks of illuminated animals is twice that from stalks of animals in darkness. In *Palaemonetes* chromatophorotropic hormone is released in darkness as shown by the concentrated state of the integumentary erythrophores, but synthesis of the hormone is either slow (indicated by the reduced hormone content of stalks from *Palaemonetes* kept in darkness) or it has ceased altogether. The latter alternative is possible if Brown (1935*a*) is correct in stating that the integumentary erythrophores of *Palaemonetes*, kept in darkness for 2-3 weeks, are dispersed; this would indicate cessation of hormone synthesis and gradual exhaustion of the hormone stored in the sinus gland. Incident light accelerates the synthesis of hormone in the gland, but inhibits release of the hormone into the circulation

(stalks from animals on an illuminated black background have as much hormone as those from animals on an illuminated white background, but no hormone is released in animals under the former conditions since the erythrophores are dispersed). The white-background response of *Palaemonetes* is due to the combined effects of incident and reflected light which cause maximum synthesis and release of hormone. There is the possibility that the pigmentary state (and the release of hormone) in *Palaemonetes* on various backgrounds may be regulated by the ratio incident light/reflected light, as Kleinholz & Knowles (1938) have already reported for the retinal pigment of *Leander*.

The physiology of secretion in *Uca* would appear to be different from that described above for *Palaemonetes*. The stalks of *Uca* which had been kept in constant darkness and in constant illumination were removed both during the day and at night and assayed by Abramowitz. The amount of hormone present in the stalks was the same whether the animals were in the pale phase or in the dark phase of their rhythm. The colour change in *Uca* is periodic only and is controlled by cyclic release of the hormone. The release is due either to a diurnal nervous stimulation of the gland or a nocturnal nervous inhibition of the sinus glands, which, if not inhibited, would continue to secrete in normal fashion.

Although no detailed quantitative studies have been made on *Ligia baudiniana*, this animal seems to combine some of the features of *Palaemonetes*, which has no persistent cyclic pigmentary activity, with those of *Uca*. In *Ligia*, maintained in darkness, there is release of hormone at night and inhibition of hormone release during the day. But illuminated animals, both during the day and at night, show characteristic chromatic responses to white and to black illuminated backgrounds, and are able to mask the pigmentary state due to the particular phase of the cyclic activity prevailing in darkness. In *Ligia baudiniana* we may therefore distinguish between a primary condition of persistent rhythmic colour activity and the secondary chromatic response to illuminated backgrounds.

Since the sinus gland shows a complicated innervation (Welsh, 1941), in all probability such cases of constant periodicity are due to cycles of activity in the central nervous system, rather than in the gland itself.

(5) *Chemical properties of the chromatophorotropic hormone*

Relatively little is known regarding the chemical properties of the crustacean eye-stalk hormone. Abramowitz (1940) has recently made the most extensive description of its nature.

The chromatophorotropic effect of eye-stalk extract is not destroyed by boiling in water (Koller, 1930; Perkins & Snook, 1931; Hanström, 1935; Kleinholz, 1937*a*), nor by boiling for brief periods in 0.1*N* HCl. Total loss of activity occurs when the extract is boiled for 2 hr. in 1% NaOH (Abramowitz, 1937*a*). From 45 to 80% of the extract is soluble in absolute ethanol and methanol; there is practically no solubility in absolute acetone, ethylether, petroleum ether, benzene, chloroform, ethyl acetate and pyridine (Abramowitz & Abramowitz, 1938).

The hormone is apparently of small molecular size, diffusing through cellophane

trations of stalk extract effected migration of the proximal pigment as well as the distal pigment. It is possible that if the stalk extract injected into *Palaemonetes* had been allowed to act a longer time in Kleinholz's experiments, migration of the proximal pigment might have been induced. In the fourth series of tests eye-stalk extracts, prepared from animals adapted to darkness and injected, in the dark, into dark-adapted *Palaemonetes*, also induced proximal migration of the distal retinal pigment, but only about half that produced by the use of extract prepared from the stalks of light-adapted animals. Control injections were all without effect on the distal retinal pigment of dark-adapted *Palaemonetes*.

This effect on the retinal pigments was not limited to extracts prepared from the eye-stalks of *Palaemonetes*; similar results were obtained with the injection of extracts prepared from the stalks of *Cancer irroratus*, *Libinia dubia*, *Carcinides maenas* and *Uca pugnator*. Only extracts from the stalks of *Callinectes sapidus*, the blue-crab, failed to induce marked migration of the distal retinal pigment. In view of the fact that stalk extract of this crab is effective in concentrating the body pigment of *Palaemonetes*, Kleinholz (1936) suggested that the retinal effectors might have a higher concentration threshold to the hormone than do the integumentary effectors (and consequently that no retinal effect was obtained because of the use of too dilute preparations) or that several pigmentary hormones are present in crustaceans and that *Callinectes* lacks the one regulating the distal retinal pigment.

The hormonal control of crustacean retinal pigment migration reported by Kleinholz has been confirmed and extended by Welsh (1939). Kleinholz & Knowles (1938) found that movement of the distal retinal pigment in *Leander* was not an 'all-or-nothing' response to conditions of illumination, but that the amount of migration could be graded between the limits of dark and of light adaptation, and that this gradation could be achieved by varying the intensity of illumination and the shade of the background. Their results showed that, provided the background was kept constant, the amount of migration of the distal retinal pigment was directly proportional to the intensity of incident light. The effect of backgrounds was shown by the different extents to which the distal pigment migrated in animals kept at the same intensity of incident light but on backgrounds of different shades. Finally, they found that the position of the distal retinal pigment stood in direct proportion to the ratio (intensity of incident light)/(intensity of reflected light).

That there is a connexion between the amount of migration of the retinal pigment and the amount of hormone secreted is indicated by further studies of Kleinholz (1938). Kleinholz found that injection of 0.04 c.c. of an extract containing 0.1 eye-stalk in 1.0 c.c. of Ringer's solution evoked a slightly perceptible response of the distal retinal pigment. This concentration of stalk extract can be labelled the minimal effective dosage for the distal retinal pigment. The amount of migration of the distal retinal pigment, at any fixed time after injection, varies directly with the logarithm of the concentration of the injected extract. Thus the graded responses observed by Kleinholz & Knowles (1938) as varying with the intensity of the incident and reflected light may be mediated by the amount of hormone released into the circulation.

The results reported by Kleinholz (1934, 1936, 1938), Kleinholz & Knowles (1938), and Welsh (1939, 1941) indicate in generally satisfactory manner the presence of an endocrine factor in the migrations of the crustacean retinal pigments. Absolute hormonal control of the activities of the retinal pigments has not yet been conclusively demonstrated. That is, the evidence at present consists only of results obtained from injection experiments. Unfortunately it is not known for certain what is the source of the retinal pigment activating substance or substances. It has been generally assumed by workers in this field that Hanström's sinus gland also secreted the retinal pigment hormones. Welsh (1941) found that aqueous extracts of isolated sinus glands would, on injection, bring about typical migration of the retinal pigments in the crayfish.

In a study of the innervation of the sinus gland and its normal physiology in retinal pigment migration, Welsh (1941) postulated two possible mechanisms: (1) Assuming that the innervation of the gland is single and excitatory, light, under otherwise normal environmental conditions, causes release of hormone from the sinus gland and consequent migration of the retinal pigments; absence of light prevents release of the hormone. But, since a number of factors, such as low temperature, oxygen-deficiency, anaesthesia, which normally lower conduction rate or decrease the activity of the nervous system, effect a partial or complete migration of the retinal pigments of crayfish kept in darkness into the position characteristic for light-adaptation, a second possible mechanism is presented. (2) The inhibition of inhibitory stimuli; this assumes that tonic impulses from the fourth optic ganglion or from the 'brain' inhibit release of the hormone from the sinus gland (the situation found in dark-adapted animals); stimulation of the eye by light causes reflex inhibition of the inhibitory centres, resulting in release of hormone from the sinus gland and consequent migration of the retinal pigments into the light-adapted position. Such factors as low temperature and anaesthesia would likewise abolish these tonic inhibitory impulses and produce effects similar to that of stimulation by light. These hypotheses can be tested by experimental methods and further discussion awaits results from such experiments.

Intimately related to the normal movements of the retinal pigments which are evoked by changes in light intensity, is the situation found in several species of crustaceans where this diurnal rhythm persists under constant environmental conditions of illumination or of darkness. Parker (1932), Kleinholz (1937*b*), and Welsh (1938) have recently summarized and reviewed such cases. This condition therefore indicates a cyclic activity within the organism.

Welsh (1930*b*) discovered that when the Cuban prawn *Macrobrachium* was kept in a brightly illuminated environment, the distal pigment moved outward into the position characteristic for a dark-adapted retina at the time of sunset. It remained in this position during the night and the following morning migrated into the light-adapted position. Kleinholz (1937*b*) reported an identical situation in the retinas of two Bermudan prawns, *Eusicyonia* and *Trachypeneopsis*. The reflecting and proximal retinal pigments in the above crustaceans showed conventional responses to light and to darkness with no persistent rhythm. In *Leander affinis*

and *Anchistioides antiguensis* (Welsh, 1935, 1936) distal and reflecting pigments during the day migrate into the light position, even though kept in darkness, and at night migrate into the position characteristic for darkness, notwithstanding constant illumination. In four brachyurans, *Portunus anceps*, *P. depressifrons*, *Parthenope serrata*, and *Calappa flammea*, Kleinholz (1937*b*) observed the light-adapted position of the distal and the proximal pigments in individuals kept in darkness during the day. Welsh (1935) found a persisting rhythm in the reflecting pigment of *Leander tenuicornis*, while the distal and the proximal pigments underwent normal photomechanical changes; in *Latreutes fucorum* both reflecting and proximal pigments showed continuing diurnal periodicity, the distal pigment responding ordinarily only to illumination or darkness. Bennitt (1932*b*) reported a persistent rhythm in the proximal pigment of the crayfish, *Cambarus*, but Welsh (1941) has found this to involve also the distal pigment. *Peneopsis goodei* (Welsh, 1935) exhibits a persistent rhythm of the proximal pigment only, the remaining pigments being fixed in position and not migrating.

These very varied responses of the retinal pigments are open to interpretation on a hormonal basis as found in the case of *Palaemonetes* (Kleinholz, 1936). In those cases where one or two of the retinal pigments show the persistent rhythm and the others undergo only normal changes in position characteristic for the particular condition of illumination, the indication is that such variations in response may be due to threshold differences to the hormone among the three sets of pigments; at the same time the conditions found in different species would show that the order of sensitivity of the three types of retinal pigments to the hormone is not constant throughout the Crustacea. A second possibility to account for the assortment of responses is that a different hormone is concerned with the regulation of each set of pigment. No decision can at present be made between these two possibilities.

It was assumed at the beginning of this section that three possible mechanisms might be involved in the regulation of the crustacean retinal pigments: the nervous system, blood-borne substances (hormones), or the retinal pigments might act as independent effectors. There has been no satisfactory evidence, either histological or physiological, that the pigment cells were directly innervated. The possibility that the retinal pigment cells act as independent effectors seems to be ruled out as a controlling mechanism by the situations found in those crustaceans in which one or more of the retinal pigments show persisting periodicity under constant conditions of illumination. Experimental evidence has supported only the theory of hormonal control. Possibly there may be one dominant mechanism with secondary 'safety' arrangements; Kleinholz & Knowles (1938) observed that in any particular stage of light adaptation the distal pigment in the dorsal portion of the eye had undergone a greater amount of migration toward the basement membrane than the pigment in the ventral portion; this might indicate an additional direct effect of light on the dorsal cells since those effectors were nearer the source of illumination. Their experiments to test this possibility were not very conclusive, and Kleinholz & Knowles decided that the situation needed further study.

IV. THE NUMBER OF HORMONES REGULATING THE PIGMENTARY EFFECTORS

The number of hormones concerned in the regulation of crustacean pigmentary effectors has become a controversial problem. One view holds that a multiplicity of different hormones are involved, and the other that a single hormone can explain all the observed effects. Koller was the first to suggest the presence of more than one pigmentary hormone. It has already been shown that there has been no confirmation of Koller's 'expantin' hormone. Smith (1938) revived the idea of two hormones in his study of *Ligia oceanica*, but his conclusions were reached on purely theoretical grounds.

Brown (1935*b*) reported that when *Palaemonetes* were kept on backgrounds of different colours the response was such that each type of pigment responded independently of the others, and consequently four separate hormones were concerned in the regulation of metachrosis of this animal. Abramowitz (1937*b*) has, however, indicated contradictions among Brown's results. Brown's data are qualitative and, although he says 'for a given background tint the state of the red, yellow and blue pigments is, within the limits of the fluctuations, quite constant', no indication is given of the range of those fluctuations. What consequently appear as inconsistencies or contradictions among Brown's data may really be the expression of wide variations among the observations.

Abramowitz (1937*a, b*) advocated the unitary hypothesis of hormonal control of metachrosis. This view resulted, in part from the observation first made by Megušar (1912) that eye-stalk removal in brachyurans resulted in concentration of chromatophoral pigments rather than the dispersal typical for the macrurans. Carlson (1935, 1936) and Abramowitz (1935, 1937) rediscovered this effect and found in addition that when extracts from the eye-stalks of *Uca* were injected into blanched (stalkless) crabs, the animals darkened. Perkins (1928) had already found that injection into stalkless (dark) *Palaemonetes* of extracts of its own stalks concentrated the erythroploral pigment. Both Abramowitz and Carlson further reported that extracts from *Uca* stalks injected into stalkless *Palaemonetes* and extracts of *Palaemonetes* eye-stalks injected into stalkless *Uca* produced the identical response obtained when the test animals were injected with extracts prepared from their own eye-stalks. Abramowitz (1937*b*) extended these observations to include reciprocal injection experiments among *Crago*, *Uca* and *Palaemonetes*. The results were such that he concluded 'either there is present in the eye-stalks of every crustacean investigated one pigmentary hormone whose effects are determined by the particular chromatophoral organization of the species into which it is injected, or that each crustacean contains in its eye-stalks a veritable array of hormones, one for but one of the two phases of a particular pigment, so that depending on the specimen injected, a certain pigment may be now contracted, now expanded'. The implication of the single hormone hypothesis is that the various integumentary chromatophores have different thresholds to the active substance. Adaptation of body tint in crustaceans maintained on coloured backgrounds may be due to the intensity of light reflected from that background, indicating that the ratio incident light/reflected light may

apply to the behaviour of the integumentary chromatophores as it does to the migration of the distal retinal pigment (Kleinholz & Knowles, 1938).

Kleinholz (1938) was able to show that the hormone affecting migration of the distal retinal pigment was probably different from that regulating the integumentary chromatophores. This distinction was indicated by the comparative behaviour of both these effectors. When *Palaemonetes* are maintained on an illuminated white background, the erythrophores are maximally concentrated and the distal retinal pigment is in the light-adapted position; on an illuminated black background the erythrophores are dispersed, but the retinal pigment is still in the light-adapted position. If both types of effectors were controlled by the same hormone, absence of the hormone from the circulation (the dispersed erythrophores in individuals on illuminated black backgrounds) should also result in complete dark adaptation of the retinal pigment. If the difference in behaviour of these two effectors is explained on the basis of differential thresholds to the same hormone, then that for the retinal pigment would need to be lower than that for the erythrophores.

Additional physiological evidence on this point comes from observations of the pigmentary behaviour of *Palaemonetes* in darkness. In this environment the retinal pigment is completely dark-adapted, indicating that no hormone controlling this effector is in the blood stream; the behaviour of the erythrophores has, until recently, been in doubt. Abramowitz (1937*a*) realized that if Brown (1935*b*) were correct in stating that the erythrophores were dispersed in darkness there would be little reason for postulating the existence of separate hormones for the retinal and integumentary pigments. But Hanström (1937*a*) confirmed Perkins's (1928) earlier report that the erythrophores are concentrated in darkness. Kleinholz (1938) subsequently determined threshold levels of distal retinal pigment and erythrophores to stalk extracts in *Leander adpersus*. The threshold for the distal retinal pigment was twenty times greater than that for the erythrophores and he therefore concluded that two separate hormones were present for the two effectors. The assay for retinal pigment hormone was conducted on intact animals while that for the chromatophorotropic hormone was done on stalkless individuals. There is the possibility, although it has not been demonstrated, that stalkless animals might be more sensitive to hormone than intact prawns.

Brown & Scudamore (1940) decided that since sinus gland extracts from seven different species showed dissimilar relative effects on the chromatophores of *Uca* and of *Palaemonetes* which were used as test animals, the effects could not be explained in terms of a single chromatophorotropic hormone. But it has already been explained in the section on localization of the source of the hormones that much of Brown's data (1940), on which the observations of Brown & Scudamore (1940) are based, were of doubtful quantitative significance.

The controversy in this field has been a constant stimulus for further research. Neither view can at present be proved to the exclusion of the opposing explanation. Until more information on the chemistry and physiology of the crustacean hormones is available, until purified extracts are tested for specific individual effects, no final decision as to the exact number of pigmentary hormones can be reached.

V. CALCIUM METABOLISM

Koller (1930) reported that after eye-stalk ablation (thereby removing the glands secreting chromatophorotropic hormone) calcium in moulted exoskeletons of *Crangon* was less than in the casts from normal shrimp. He also found the calcium content of exoskeletons from white-adapted animals to be higher than those from black-adapted individuals and therefore stated that metachrosis and calcium metabolism were related processes, regulated by the same eye-stalk organ. Plankemann's (1935) similar conclusion was inferred from the known hormonal regulation of moulting in insects and from the fact that the calcium content of the crustacean skeleton was related to the moulting process.

Kleinholz (1941) found no significant quantitative difference in calcium content between casts of normal *Palaemonetes vulgaris* and those of eye-stalkless individuals. In Koller's assays the cast skeleton, after being measured in length, was dried to constant weight. The dried moult was immersed in 10% HCl for 24 hr., after which it was washed, dried and weighed. The difference between dry weights Koller designated 'Kalkgewicht'. These values for calcium were plotted against size classes determined by the length of the cast. Plankemann used the same method. Rather wide variation among the results raises the question of the validity of conclusions based on such measurements.

The relation between calcium and moulting in Crustacea has been studied by several investigators (Hecht, 1914; Paul & Sharpe, 1916; Numanoi, 1934, 1939; Drach, 1939; Kleinholz, 1940, 1941). Certain deposits rich in calcareous material (the gastroliths and the hepatopancreas) have been suggested as internal reserves from which calcium for the new skeleton was drawn.

Hecht showed, however, that once the skeleton of *Callinectes* was cast, the amount of calcium present in the newly-moulted individual was insufficient for the rebuilding of the new skeleton. Paul & Sharpe (1916) reported that calcium was withdrawn from the hepatopancreas after ecdysis, so that when the new shell was hardened, calcium was almost absent from this organ. The amount of calcium stored in this gland is insufficient for the complete reconstruction of the skeleton and therefore indicates an additional external source of calcium. Kleinholz (1941) found that *Uca pugnator*, killed within 5 min. after ecdysis, contained calcium equal to 1% of the dry body weight; this internal reserve constituted only 6% of the total calcium content of the normal intermoult crab. The balance of the calcium content was absorbed from the sea water after ecdysis.

The calcium which is found as an internal reserve in newly-moulted decapods, although inadequate for the complete restitution of the new skeleton, may be an adaptive device whereby initial hardening of the newly formed skeleton is facilitated until sufficient mineral material can be absorbed from the sea water. This seems to be indicated by the observations of Paul & Sharpe and by those of Numanoi (1939). The latter investigator found that the gastroliths of *Sesarma haematocheir* enlarged as ecdysis neared and disappeared after the moult; these changes were correlated with periodic fluctuations in the level of blood calcium, indicating mobilization and

transport of calcium from the exoskeleton to the gastroliths before ecdysis and in the reverse direction after moulting.

The intimate relation between ecdysis and the transfer of this reserve calcium indicates that both processes may be mediated by the same mechanism. A hormonal factor in moulting among Crustacea has been suggested by the work of Brown & Cunningham (1939). Although the connexion may be more complicated than is apparent, a speculative interpretation would connect the inhibition of ecdysis with retention of calcium in the exoskeleton; absence of the hormone would initiate moulting and migration of calcium to the internal depot; transfer of calcium in the reverse direction after completion of ecdysis might be mediated either by the re-appearance of the same hormone in the circulation or by some other agent.

VI. VIABILITY HORMONE

Brown (1938) and Brown & Cunningham (1939) reported a 'viability effect' in crayfish on removal of both eye-stalks. They believed a hormone, produced in the eye-stalks, was essential to continued life of the animal, although they recognized that this function was described in too general terms. According to these authors, removal of both eye-stalks shortened the life span of their animals while implantation of Hanström's sinus gland lengthened the survival of stalkless animals over control groups. Abramowitz & Abramowitz (1940) found a total mortality of 89 % in 48 days in eye-stalkless *Uca pugilator*, thereby apparently confirming in part the observations of the former investigators.

In contrast with these reports are the records of Herbst (1902) and of Megušar (1912) in which several species of crustaceans lived for months and even years following ablation of the stalks. Kleinholz & Bourquin (1941), in a study which essentially duplicated that of Abramowitz & Abramowitz, found a mortality of only 22 % in stalkless *Uca* at the end of 40 days; 10 % of the control crabs had died during this period.

The observations of these latter two groups of investigators are sufficiently different to merit further analysis. A. & A. found a striking similarity between the mortality and moulting curves of their experiments which gave the superficial impression that the two processes were related. 70 % of the total mortality occurred during or within 1 day of moulting. K. & B. noticed that those crabs which were among the first to moult after eye-stalk ablation, died because of difficulty in completing ecdysis; the soft, half-emerged crabs hung limply over the edge of the carapace, unable to withdraw the large chelae and mouthparts. When sufficient sea water was added to the containers to cover the animals to a depth about 1 cm. above the carapace, the moulting individuals were buoyed up, and no further deaths during ecdysis occurred.

The results of Brown & Cunningham may, in part, have been influenced by a similar mechanical factor, since their Table 1 shows that 44 % of their normal crayfish died in the process of ecdysis. The possibility that some additional environmental agent was influencing viability in their experiments is indicated by their

report that normal, unoperated crayfish survived, on the average, only 13 days. The animals were not fed, but according to Brunow (1911) crayfish can live for months without food.

Brown (1938) and Brown & Cunningham (1939) reported that implantation of Hanström's sinus gland into eye-stalkless crayfish increased the life-span of these animals and was therefore proof of an endocrine control of viability. Brown's Table 1 reports the survival of unoperated animals and of eye-stalkless crayfish both with and without implants of stalk tissue. In the second paper (B. & C.) no direct data are given on viability, but this information can be obtained from their Tables 1 and 2, and by analysing the mortality curves of their Figs. 2 and 3. Comparison among these data reveals inconsistent results, so that the explanation of a hormonal influence on viability must be considered inconclusive. The fact that their normal unoperated crayfish lived only 13 days indicates that some environmental factor was influencing their results. It is unfortunate in this respect that their mortality curves, which served as controls for the experiments, were based on only six and seven individuals.

If the high mortality of eye-stalkless *Uca* reported by Abramowitz & Abramowitz was due entirely to the factor suggested by Kleinholz & Bourquin, then the 20% mortality among the eye-stalkless animals is not sufficient basis for postulating hormonal control of viability. In all three sets of experiments (B. & C.; A. & A.; K. & B.) ablation of the eye-stalks removed not only Hanström's sinus gland but also the four optic ganglia in each stalk. The third and fourth optic ganglia constitute a considerable portion of the cephalic nervous system and the mortality may indeed have been the consequence of this injury to the animals.

VII. MOULTING

Megušar (1912) observed that ecdysis occurred earlier in blinded *Astacus* than in normal crayfish, while Abramowitz & Abramowitz (1938, 1940), Brown & Cunningham (1939) and Kleinholz & Bourquin (1941) noted similar effects on moulting in several different species of crustaceans after removal of both eye-stalks. Smith (1940) placed this observation on a firm quantitative basis by showing that ablation of both stalks in young crayfish shortened the intermoult period by slightly more than 30%.

The mechanism for this shortening of the intermoult period has not been incontrovertibly demonstrated. Darby's (1938) statement that operative injury appeared to hasten the onset of the ensuing moult in *Crangon armillatus*, and Plankemann's (1935) report that nearly 80% of the total number of moults in *Crangon* occurred within the first 6 days after the animals were brought into the laboratory may indicate a mediating nervous agency. Smith's control experiments, however, definitely exclude injury as a general explanation of this situation. Furthermore, in several experiments (A. & A.; K. & B.; Smith) eye-stalkless crustaceans moulted several times long after 'shock' effects of the operation could be presumed to have worn off.

Plankemann (1935) reported that such diverse factors as starvation, colour of the background, increased calcium, and pH-concentration of the sea-water may affect the rate or amount of moulting. The same author's conclusion that moulting in crustaceans is under hormonal control is based on no direct evidence but merely on the analogy with ecdysis in insects (Wigglesworth, 1934).

Brown & Cunningham (1939) have been the only investigators to make direct experimental test of the possibility of hormonal control of ecdysis in crustaceans. The results of their observations led them to conclude that a hormone from the sinus gland normally inhibited moulting, that when the gland was removed through ablation of the eye-stalks the rate of ecdysis increased; conversely, when sinus glands were implanted into eye-stalkless crayfish, moulting activity was delayed. The experiments of these investigators were partly complicated by low survivals of their animals, so that while an endocrine factor in the regulation of ecdysis is indicated, more detailed evidence for this view is lacking.

VIII. SEX HORMONES IN CRUSTACEA

(1) *Parasitic castration*

Modifications in the secondary sexual structures of crustaceans have long been known, but the mechanism whereby such changes were effected have not been clearly understood. Various investigators have reported observations that indicate the possibility of sex hormones as agents in the maintenance of secondary characters. The evidence comes from two principal sources, studies of parasitic castration and from castrations induced by X-ray and radium treatment.

Giard (1886-8) was the first to describe the effects of *Rhizocephala* and other parasites on crustacean hosts. The young of *Sacculina*, hatched as nauplii, are for a short period free-swimming before attaching to a host, usually a crab. Hyphe-like branches that penetrate the host and spread over the internal organs, absorbing nutriment from them, finally form a rounded mass which lies externally on the ventral abdominal surface of the crab. Later investigators (Smith, 1906-13; Potts, 1906; Tucker, 1930; Caroli, 1932; Day, 1935; Okada & Miyashita, 1935; Brinkmann, 1936) studied the effects of parasitic forms on various crustaceans. These reports demonstrated differences in the degree of modification of the host, depending in part upon the parasite and in part on the diversity of the secondary sex characters among the members of the host species.

Giard found that in sacculinized male crabs the claws, pleopods and abdomens assumed the appearance characteristic of female crabs, while the testes showed more or less atrophy; infected female crabs showed relatively little change.

Okada & Miyashita (1935) described the effects of sacculinization in *Eriocheir*. Infected females showed no change toward the male condition. In parasitized males the modifications varied from slight reduction of the copulatory styles to complete development of female appendages, entire absence of copulatory styles, and wholly feminized abdomens. The ovaries of parasitized females were almost

completely destroyed by *Sacculina*; if incompletely destroyed, they were reduced to inconspicuous bodies; in several cases, however, these authors found apparently normal ovaries. In lightly-parasitized males the roots of *Sacculina* penetrated the testicular tissue, causing reduction and separation of the seminal tubules; these tubules contained normal spermatocytes. Degrees of hermaphroditism ranging to complete sex reversal were found among highly parasitized males. There was, however, no definite correlation between the degree of modification of the gonads and the external secondary sex characters of these crabs.

Brinkmann (1936) studied the effects of three species of Rhizocephala on the decapod, *Munida*. *Triangulus munidae* caused marked atrophy of the host's gonads, but in some males normal spermatozoa were present in the tubules. *Lernaeodiscus ingolfi* produced only a partial decrease and *Triangulus boschmai* little destructive effect on the host gonad. The first two rhizocephalans reduced the copulatory pleopods in male *Munida* and caused the third, fourth and fifth pleopods to develop in a female direction.

Several explanations have been suggested for the effects of parasitic castration on the secondary sexual characters. Smith (1910-13) postulated the existence in the blood of 'sexual formative substances', male and female, which determined both the development of the gonads and the secondary sex characters. According to this author, the ovary required large amounts of fatty material to build the yolk; the female sexual formative substance was both a stage in the formation of the food material for the ovary as well as a substance on which the development of the female secondary characters depended. In sacculinized males the concentration of these fatty materials in the blood of the host was lowered to a point where the demand for these substances altered the host metabolism to the female type and thus resulted in the development of female secondary characters. That is, the parasite assumed the same role in fat metabolism normally played by the maturing ovary. Smith showed that the amount of fat in the blood and liver of sexually mature females differed from that in males, and that parasitized individuals developed a comparable excess. Hughes (1940) has confirmed these observations. Smith's hypothesis identified the changes in the secondary characters with metabolic activity, and is in contrast with the idea of control by a hormone produced by the gonad itself.

Lipschütz's (1924) explanation was that a common neutral form resembling the female exists among crustaceans; normally the testis inhibits female secondary characters and evokes those of the male, but parasitic castration removes this inhibition and allows female characters to develop. This view encounters certain difficulties. Okada & Miyashita found that the abdomen of the young crustacean resembles the male rather than the female type until the crabs reach a carapace length of 40 mm.; from this stage onward the female abdomen assumes the trough-like adult shape. The same investigators also observed no close correlation between the presence or absence of the gonads and the modification of the external secondary sex characters.

Goldschmidt (1931) believed the results of parasitic castration had no causal relation with sex hormones of the host, but that the quantitative balance between

male and female 'sex formative substances' present in each individual was disturbed by the parasite, resulting in the characteristic modifications.

Biedl (1913) suggested that the parasite not only destroyed the gonads of the male host but also acted as a transplanted ovary, since he believed *Sacculina* to be female. Although the sexual functions of *Sacculina* are incompletely known, Smith (1906) reported it to be hermaphroditic, so that Biedl's explanation is not readily acceptable.

According to Brinkmann (1936) the changes in parasitized males were due to both hormonal and nutritional factors. He believed male hormone not only maintained the secondary characters but also inhibited the appearance of female characters. Parasitic destruction of the testes coupled with pronounced inanition effected changes in the secondary structures.

Clarification of the effects of parasitic castration are evidently difficult. For, even if sex hormones are concerned, the marked metabolic disturbances introduced by the presence of the parasite make this evidence not wholly conclusive. A second difficulty is that little is known regarding the frequency of sex reversal among the decapods (Runnström, 1925). Turner (1935) has postulated that a hermaphroditic condition once existed in the genus *Cambarus* and that 'the occasional occurrence of oviducal pores in the males and of copulatory hooks and male-like first and second abdominal appendages in females represents an atavistic reappearance of these characters. An alternative point of view... is that the determiners for these characters have... become fixed in the germ plasm of the opposite sex.'

(2) Radiation castration

The second method of investigating sex hormones in Crustacea is uncomplicated by a host-parasite relationship. Haemmerli-Boveri (1926) exposed female *Asellus*, in which cyclic formation of the brood-pouch is correlated with the condition of the ovary, to radium emanations that destroyed the ovaries but caused no other apparent injury to nearby organs. In such irradiated females the ability to form brood-pouches was subsequently lost. Le Roux (1933) similarly irradiated female *Gammarus* and found that the ovigerous hairs on the oostegites later failed to develop and that egg-laying was inhibited; the ovaries were not destroyed but only retarded in function. Le Roux considered this evidence for an ovarian hormone regulating the secondary sex structures. It is equally possible, however, that the radiations acted directly on the secondary structures rather than by inhibiting an ovarian hormone. Radium castration of *Daphnia magna* by Mori (1933 *a, b*) led to inconclusive results; there was no effect on the male secondary structures, while in females formation of the brood-pouch and other secondary characters was prevented. Mori reported, however, that these castrations were accompanied by extensive injuries of somatic tissues, thereby influencing the metabolism of such animals.

Knowles & Callan (1940) described a new secondary sex character in prawns of the genus *Leander*. Mature females developed clusters of white chromatophores on the pleopods of the egg-bearing segments during the breeding season. From

1 to 3 months before the beginning of the breeding season, females were subjected to X-ray treatment that inhibited vitellogenesis and destroyed all the oocytes. Control females developed the clusters of guanophores in the usual location, but the irradiated animals failed to do so. These authors reported that such chromatophores were also lacking in females whose ovaries had been inhibited by bopyrid parasites. The appearance of these guanophores was not considered proof for a female sex hormonē; K. & C. believed that the white pigment (presumably guanine) was 'related simply to the metabolic conditions (high nucleoprotein turnover) which obtain during yolk deposition'.

Callan (1940) reinvestigated the effects of bopyrid parasitization and radiation castration on the secondary sex characters of *Leander*. Male structures such as the copulatory styles and the first two pleopods varied considerably in size. There was no significant difference in the size of these structures between parasitized and unparasitized males, even when extreme reduction of the gonad had occurred in the former group. X-irradiation of females before the breeding season destroyed the oocytes; individuals that survived the pre-breeding moult failed to develop the secondary breeding characters that appeared in the controls. Callan did not accept these results as conclusive proof of a female sex hormone, but suggested that radiation might have acted directly on the breeding structures or that the effect might be due to depression of the general metabolic level induced by radiation injury.

Morgan (1920) attempted to test directly for the presence of gonadal hormones in crustaceans. Male and female *Uca*, whose large claws had been removed, were fed crustacean ovarian and testicular tissue respectively and were observed for several months during which they moulted normally; the regenerating appendages were unaffected by the nature of the food. Implantations of gonad tissue into crabs of the opposite sexes produced no effect on the claws.

It has been extremely difficult to prove conclusively the presence of crustacean sex hormones from the studies cited above. Parasitic and radiation castration are not wholly desirable methods of analysis of this problem because of the generalized metabolic complications introduced. Surgical castration has, up to this time, been almost impossible, presumably because of the mechanical difficulties encountered. More convincing proof, unencumbered by gross metabolic disturbances, may come from injection of extracts prepared from the gonads of the two sexes.

IX. SUMMARY

Colour changes in crustaceans are controlled by a secretion originating in the eye-stalk. The glandular tissue is probably Hanström's sinus gland, although the X-organ may also be concerned in this function. Retinal pigment migration is similarly regulated by a hormone from the eye-stalks. Physiological evidence points to the existence of at least two pigmentary hormones, one for the chromatophores and another for the retinal pigment. No conclusion can yet be made concerning the exact numbers of these hormones. Cyclic activity of these pigmentary effectors under constant environmental conditions is explained on a nervous-hormonal basis.

There is no evidence that calcium metabolism is under specific hormonal control. The reserve pre-moulting calcium may be regulated by an eye-stalk hormone that is also suspected of inhibiting ecdysis. The viability effect reputedly regulated by eye-stalk hormone may be due to nervous injury rather than to a specific active substance.

Maintenance of the secondary sex characters has been thought to be controlled by the sex hormones. The evidence, coming chiefly from studies of parasitic and radiation castrations, is not wholly reliable because of metabolic disturbances introduced under these conditions. Direct evidence from injection of glandular extracts is lacking.

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THE CO-ORDINATION OF VERTEBRATE MELANOPHORE RESPONSES

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I. INTRODUCTION

INVESTIGATIONS of chromatic responses up to 1924 were critically reviewed by Hogben (1924). This and later articles by Parker (1930) and by Sand (1935), both in *Biological Reviews*, deal adequately with the early history of the subject. The present review is concerned with recent work on the co-ordinating mechanism of chromatic response, but it will be necessary at the outset to draw attention to certain important findings regarding the effector and receptor mechanisms.

(1) *Effector system*

Colour response in vertebrates has been described only in the cold-blooded species belonging to the classes Cyclostomata, Pisces, Amphibia and Reptilia. In these groups colour change is brought about by the 'contraction' or 'expansion' of dermal and epidermal chromatophores. According to the pigment they contain, chromatophores are distinguished as melanophores, xanthophores, erythrophores, etc. According to situation they are described as epidermal or dermal. The melanophores, especially the dermal melanophores, are generally the most important in the change from a dark to light condition.

The terms *contracted* and *expanded*, used by early workers who believed that chromatophores were amoeboid, are retained in accordance with custom. An

alternative view is now generally accepted. Conclusive evidence for it has been furnished by observations made on *Fundulus* by Matthews (1931), who used the technique of tissue culture, and was able to distinguish clearly a fixed cell boundary unaffected by the migration of the pigment granules within it. By use of magnifications up to 1000 diameters Herrick (1933) was able to discern the same structure in frog tadpole melanophores. To describe these states more accurately the terms *dispersion* and *concentration* of pigment have been used by some authors.

(2) *Melanophore index*

Early workers were content to describe chromatic behaviour in terms of the macroscopic appearance of the animal, as dark, intermediate, pale, etc. Parker and his co-workers still adhere to this method. Descriptions of this kind are open to several objections. The terms used are inexact. Hence the records of different workers are not comparable, and communications are verbose. A more important objection which also applies to the method of Hill (*vide infra*) is that macroscopic appearance depends on several different microscopic organs and on the previous history of the animal. On the other hand, descriptions of individual systems of pigmentary effector organs can be more exact. Hogben & Winton (1922-3) used

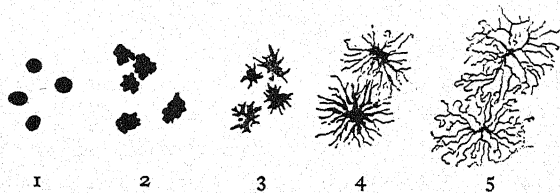


Fig. 1. The melanophore index. (Hogben & Slome, 1931.)

punctate, stellate and reticulate to denote the condition of the melanophores. Hogben & Gordon (1930) introduced a numerical index in which unity stands for the fully contracted condition and 5 is assigned to the fully expanded condition (Fig. 1). This index has been subsequently used in all publications from Hogben's laboratory. Without its use analyses of the type cited on p. 128 are not possible.

The observation of the melanophore index (μ) of teleosts is difficult. The natural change is fast, and handling the fish interferes with the direction and speed of the change. There have been three attempts to solve the problem. The first (Hill *et al.* 1935) discards direct estimation and uses a photoelectric method. Its shortcomings have been outlined by Hogben (1936*b*), Wykes (1937) and Neill (1940). Wykes (1937) has resorted to metrical measurements of *fixed* melanophores. Observation on fixed material is unsatisfactory for reasons pointed out by Hogben. With slowly reacting chromatophores which are not appreciably affected by handling, fixation causes contraction of fully expanded melanophores from 5 to about 4 on Hogben's scale. Similarly a fully contracted melanophore ($\mu = 1$) is slightly more expanded ($\mu = 2$) by the action of even a quick-acting fixative like Bouin. This is especially important in connexion with information concerning relative importance

of humoral and nervous control. Where direct innervation is superimposed upon a more archaic humoral control, the existence of a protracted asymptotic region of the time-melanophore index graph between 4 and 5, and between 1 and 2, may suggest persistence of the older mechanism.

Hogben & Landgrebe (1940) and Neill (1940) used a statistical refinement for their work on the stickleback, *Gasterosteus*, and on *Salmo*. A new batch of six animals was used for each point on a graph recording the course of colour change. In this way each point on a graph recording chromatic behaviour is based on animals which have not been previously subjected to tactile stimulation.

(3) *Primary and secondary responses to light*

Chromatic behaviour of vertebrates may be elicited by a variety of physical agencies including light, humidity, temperature, mild nocuous stimuli or intensive electrical stimulation of specific receptive areas such as the cloaca and roof of the mouth of the chameleon. The relative importance of these several agencies is not the same in different species of one and the same taxonomic group. Broadly speaking, however, light is most important and very commonly predominates over all other natural agents in vertebrates and Crustacea just as light predominates over all other agencies which influence bodily orientation of animals that are highly phototactic. In studying both phenomena the effect of other agencies can be shown by removing the eyes or eliminating light.

Two classes of melanophore response to light are commonly distinguished. The distinction was first emphasized by Laurens (1915) and later explored by Hogben & Slome (1931). A *primary* or direct response involves the expansion of melanophores in bright light and their contraction in darkness. A *secondary* or visual response involves melanophore contraction if the visual field is coincident with a light scattering surface and melanophore expansion if the animal is exposed to *superior* illumination in surroundings which do not reflect or scatter light coming from above. The eye is *not* the receptor for the primary response. It might be better to refer to non-visual rather than primary or direct response, because there is some dispute about whether the non-visual response is always direct. The work of Hogben & Slome (1931) shows that the amphibian primary response is independent of the central nervous system and so is autogenous. The work of Young (1935) on the pigmentary effector system of the ammocoete shows that the primary response is a co-ordinated one for which the receptor organ is the pineal. From their work on the chameleon, Zond (Sand) & Eyre (1934) concluded that the primary response is a reflex dependent upon skin receptors. The existence of photoreceptors in the skin of Amphibia has been independently established by the work of Parker (1903) on bodily movement. At present the evidence relating to the nature of the primary response from this point of view is unsatisfactory; it is summarized in Table 1.

To the species referred to in the table we might add the teleost *Tautoga* (D. C. Smith, 1939). Smith's work was done on isolated scales, and the response was only a temporary darkening on exposure to light. In any case Kleinholz (1938*b*) has recently shown that the behaviour of isolated skin may be an unreliable guide

Table 1. Direct and co-ordinated non-visual melanophore responses

Animal	Independent effector	Evidence	Authority	Co-ordinated non-visual response	Evidence	Authority
mmocoetes of <i>Lampetra</i>	-	Skin of hypophysectomized animals is permanently pale under all circumstances	Young (1935)	+	After removal of pineal complex animals remain dark under all conditions of illumination	Young (1935)
lasmobranch spp.	?	Blinded hypophysectomized <i>Mustelus</i> darkens in light, pales in darkness Blinded hypophysectomized <i>Mustelus</i> show no response to light and darkness	Parker (1937) Abramowitz (1939)	+	Eyeless but otherwise intact <i>Raia</i> equilibrate at 3.0-3.5 according to lighting conditions Eyeless <i>Mustelus</i> darken in light and pale in darkness	Hogben (1936a) Parker (1937), Abramowitz (1939)
<i>meiurus</i>	-	Denervated skin remains permanently dark under all conditions of illumination	Wykes (1938)	+	Blinded animals with nervous system intact pale in darkness: darken in light	Wykes (1938)
<i>enopus</i>	+	Response evoked after destruction of central nervous system and removal of pituitary gland. Magnitude: about 0.5 on the melanophore index scale	Hogben & Slome (1931)	Probably +	Response of eyeless but otherwise intact animal operated on at least 5 years previously. Magnitude: animals equilibrate at 2.5 in darkness and at 5.0 in light	Landgrebe (unpublished)
<i>irynosoma</i>	+	Denervated regions of skin on hypophysectomized animals react to light intensity	Parker (1938)	?	None	...
<i>solis</i>	-	Skin of hypophysectomized animals is permanently pale under all circumstances	Kleinholz (1938a)	+	Blinded but otherwise intact animals change from completely pale to dark when brought from the dark-room into light	Kleinholz (1938)
<i>phosaura</i>	-	Denervated skin remains permanently dark under all circumstances	Zoond & Eyre (1934)	+	Eviscerated decapitate preparations react to light only if nervous pathways are intact	Zoond & Eyre (1934)

as to what occurs in the intact animal. Working with the reptile *Anolis*, Kleinholz was able to confirm Hadley's (1928) observation that isolated skin shows some measure of direct response to light; but this was not apparent on intact skin of hypophysectomized animals.

The eye is the receptor for the secondary response. Enucleated animals, or those with the optic nerves cut, show only a primary response. In describing experiments on the secondary or visual response, two terms which are not precise in a literal sense are commonly used to avoid periphrasis. The expression 'white background' signifies that the surroundings of an animal scatter light coming from above in all directions. A 'black background' signifies an environment in which an animal is exposed to illumination from *above* alone because it is otherwise surrounded by surfaces which absorb light. Needless to say, the description of an environment in these terms may be misleading, when the eyes of an animal placed in a small vessel with a flat bottom are dorsally placed as in flat fishes.

Apparently the photic response of most vertebrates and crustaceans is a compromise between the two types of reaction specified in the preceding paragraphs, though the relative importance of the one or the other differs greatly in different species. The separate contribution of direct and visual stimulation can be dissected by several methods of which the two principal are: (a) comparison of eyeless animals in darkness and at different levels of illumination; (b) comparison of background response of intact animals at different levels of illumination.

These two classes of experiment may not discriminate completely between several possibilities. The effect of dim and bright light on intact animals may be due to differential sensitivity within the retina itself. This can be checked by parallel experiments with eyeless animals. In eyeless animals we may be dealing with two kinds of non-visual response (p. 122).

The threshold for the primary is usually much higher than the threshold for the secondary response. When suitable correction is made, it is a general rule that animals kept in darkness are paler than if kept on an illuminated black background. Thus black background response is not merely the absence of a single stimulus involved in the white background response. It can only be due to the *localization of visual stimuli*. On an illuminated black background all the light entering the eye of an aquatic animal is contained within a cone whose half-angle is the critical angle for water and air.

This possibility that white and black background responses are evoked by stimulation of separate retinal areas was first tested by Keeble & Gamble (1904) with inconclusive results. Hogben & Slome (1936) and Hogben & Landgrebe (1940) were able to establish its truth for *Xenopus* and *Gastrosteus* respectively, by methods using inferior illumination and reaction to monochromatic light. At Hogben's suggestion H. Smith (1938) showed that the same interpretation is applicable to Crustacea. Sumner (1933) approached the problem by covering the cornea of *Fundulus* with celloidin 'variously painted with opaque areas of Indian ink'. When the lower half of the cornea was covered, the fish were maximally dark on any illuminated ground. Von Frisch (1911) had previously obtained similar

results with trout by the use of opaque adhesive material, but this only stayed in place for about 15 min. Butcher (1938) was able to turn the eye of *Fundulus* in its orbit so that the ventral part of the retina was in a dorsal position. He concluded that stimulation of that part of the retina which is normally in a dorsal position causes contraction, and stimulation of the ventral part expansion.

During ontogeny a primary response may be discernible before the secondary. The paradise fish, *Macropodus* (Tomita, 1936), has a few days of free existence during which only a primary response can be detected. Background response may not occur in the trout, *Salmo trutta* (Neill, 1940), till 3 weeks after hatching. These observations may be taken to indicate that these fish are blind when first hatched. Neill has confirmed that development of the eye in *Salmo* remains incompleting for some time after emergence from the egg. In contrast, Parker (1936*a*) records that newly born smooth hounds, *Mustelus*, behave like adults. The same is true of newly born guppies, *Lebistes* (Tomita), newly hatched dogfishes, *Scyllium* (Waring, unpublished), and newly hatched *Xenopus* (Landgrebe, unpublished).

Light is not the only natural stimulus to the response of melanophores. Humidity is an agent of colour change among terrestrial Amphibia, e.g. *Rana*. Both in the latter and in reptiles (*Chameleo*, *Phrynosoma*) temperature is important. The high temperature effect on frogs was analysed by Hogben (1924). The influence of humidity has not been analysed extensively. Biedermann (1892) considered that the appropriate receptive area of tree frogs is in the toes. This work has never been repeated.

II. CO-ORDINATING MECHANISMS

(1) General

Co-ordination of visual chromatic response may be humoral or nervous or both. In this context *humoral* implies reflex stimulation of an endocrine organ. Satisfactory evidence for humoral control so defined includes *both* measurement of response to removal of endocrine organs and demonstration of complete replacement by physiological doses of endocrine extracts. Accumulative indirect evidence pointing to the existence of a hormone may be very convincing, even when it is impossible to prepare an active extract. The only acceptable evidence for direct nervous control is *reversible* response of effectors to stimulation after stoppage of the circulation. Nerve section or stimulation with the circulation intact cannot provide unequivocal evidence because of attendant vasomotor effects.

In 1924 Hogben first drew attention to the fact that where melanophores are under humoral control, colour change is relatively slow. Where there is nervous control, it is fast. There are clear reasons for this difference. A complete cycle of colour response involves: (a) time taken for stimulus to act on receptor, (b) time taken for propagation of the disturbance through the co-ordinating mechanism, (c) time taken for the effector to execute its response. If the mechanism is a purely nervous one, (b) involves: (i) transmission of the nervous impulse along the nerve trunks concerned, (ii) delay at synapses, (iii) delay at the neuro-effector junction. When the eye is the receptor, a maximum time value for (a) and for (i), (ii) and (iii)

taken all together is at the most a matter of seconds. So when the process of co-ordination is wholly nervous, the maximum time for a complete cycle, apart from the time taken by the effector to execute its response, is under a minute. Any excess is the reaction time of the effector. This can be assigned a maximum time value on the basis of perfusion experiments (Osterhage, 1932; Fenn, 1924; Spaeth & Barbour, 1917; Hogben & Landgrebe, 1940; Waring & Landgrebe, 1941; Waring *et al.* 1941). Experiments with drugs and pituitrin have shown that melanophores react rather slowly, taking 3–90 min. A total time change of the same order as the effector time is therefore consistent with nervous co-ordination through the direct innervation of the melanophores themselves. A total time of 2 hr. or more shows that some *other* mechanism is also involved, e.g. the *reflex* liberation of hormone and its gradual accumulation in the blood. Thus we may picture the process of humoral co-ordination in two stages: (a) the reflex liberation of a hormone, (b) the distribution of the hormone via the blood to the pigmentary effector.

It is thus pertinent to distinguish between *fast* and *slow* colour change. The latter signifies that a humoral agency is involved. Investigation of the time relations of natural change using the melanophore index can indicate direct nervous control for co-ordination of stimulus and response. Alternatively, it can indicate that co-ordination is brought about by the distribution of a hormone liberated by the initial reflex activation of a gland of internal secretion. It can also furnish a clue to: (i) the number of hormones concerned (p. 128), and (ii) the role of direct innervation and hormonal control when both participate in the result (p. 136).

The speed of response is also instructive if we take into consideration a distinction between two types of co-ordination invoking reflex liberation of hormones. Some endocrine organs, e.g. the pars intermedia of the pituitary, the adrenal medulla and the thyroid, *may* contain quantities of their respective hormones in excess of the total blood content. Under some conditions the pars intermedia does not contain excess of stored secretion. The storage capacity of other endocrines, e.g. the glandular elements in the vertebrate gonads, is always relatively small. If a gland contains little stored secretion, reflex activation excites (a) production and (b) liberation of secretion. This will take longer than (b) alone.

A large portion of the literature on chromatic response effector systems concerns co-ordinating systems. Publications from the laboratories of Hogben and Parker show there is not complete agreement between the two. The disagreement is not so great as appears at first sight. There is general accord on broad issues. Both agree that there is pituitary co-ordination of amphibian responses. Both agree that co-ordination of background responses of teleosts such as killifish and minnows is predominantly nervous. The divergence of opinion in regard to details is due to (a) different interpretations of the same experimental data and (b) concentration on different classes of experiment.

Differences of interpretation will be discussed in the appropriate context. In regard to (b), Hogben and his co-workers have emphasized the significance of three classes of experiment that have been largely neglected by Parker's school. They are: (a) time relations of chromatic response (pp. 128, 136), (b) responses evoked by the

separate removal of the various lobes of the pituitary (p. 130), and (c) differential tolerance of complete and partially hypophysectomized and intact pale animals to injections of pituitary extract (p. 131).

Parker and his co-workers have concentrated their attention on the following: (a) blood transfusions (pp. 134, 135), (b) behaviour of denervated melanophores (p. 140), and (c) injection of tissue extracts to test for the presence of neurohumors (p. 145).

There is reason to believe that humoral control of background responses predated direct nervous control. So the former will be considered first.

(2) Humoral co-ordination

Early workers on Amphibia did not obtain clear evidence of direct nervous control (summary in Hogben, 1924). P. E. Smith (1916) observed that hypophysectomized tadpoles are permanent albinos. Hogben & Winton (1922-3) initiated a series of investigations on the relation of pituitary gland to chromatic function of *Rana*. Their chief findings were as follows:

(i) Colour change in response to background reversal is a slow process taking hours or days.

(ii) After removal of the whole pituitary (except pars tuberalis) animals are pale in any environment.

(iii) Removal of the anterior lobe alone does not prevent expansion and contraction of melanophores under appropriate stimulation.

(iv) Injection of posterior lobe extracts evokes expansion of melanophores. There is sufficient melanophore hormone in one frog gland to darken about fifty pale frogs.

(v) Nerve section and stimulation produce no effects that cannot be attributed to vaso-motor action.

In summarizing this work Hogben (1924) claimed that the 'hypothesis of pituitary secretion fluctuating in correspondence with the action of natural stimuli tending to promote colour response is in the existing state of knowledge adequate, at least in adult Amphibia, to interpret all the salient facts'. In a recent paper on *Rana*, Parker & Scatterty (1937) reached the same conclusion. They were able to detect the presence of pituitary excitant substance in the circulation of dark animals.

Kleinholz (1938*a, b*) investigated responses of *Anolis* to nerve section and stimulation, hypophysectomy and injection of pituitary extract. His results show that the melanophores are not directly innervated. He concluded that background responses are co-ordinated by fluctuation in secretion of the posterior lobe pituitary.

The first detailed description of investigations on *Xenopus* was published by Hogben & Slome in 1931. The results were in many respects similar to those obtained during earlier work on *Rana*. Colour response of the intact animal is protracted. Nerve section and stimulation evoke no melanophore response. Hypophysectomized *Xenopus* like hypophysectomized *Rana* is pale irrespective of lighting conditions. It is temporarily darkened by injection of posterior lobe pituitary extracts.

When Hogben's monograph (1924) was written there was no reason to suppose that the co-ordinating mechanism of adult Amphibia involved more than the fluctuating secretion of one pituitary hormone (i.e. the *B* substance of Hogben & Slome from the posterior lobe). In later investigations on *Xenopus* Hogben & Slome (1931) introduced several new methods of analysing chromatic response. Quantitative study was achieved for the first time by the use of the melanophore index. The result was the formulation of a scheme involving antagonistic pituitary autacoids.

Hogben & Slome concluded that the pars tuberalis secretes a melanophore contracting hormone *W* antagonistic to the expanding hormone *B* from the posterior lobe. They did so for the following reasons:

(a) Eyeless animals equilibrate at $\mu = 2.5$ in dim light. 'The fact that eyeless animals are neither completely pale nor completely dark suggests that the white background and black background response involve separate agencies for the co-ordination of stimulus with response' (Hogben & Slome, 1931).

(b) Hogben & Slome (1936) showed that there is a separate localization of visual receptor elements for (a) white and (b) black background responses. They did this by the use of inferior illumination and of monochromatic lighting. Stimulation of basal retinal elements evokes melanophore expansion, stimulation of peripheral or peripheral and basal elements evokes contraction.

So if there is only one hormone *B*, stimulation of floor elements reflexly excites liberation and stimulation of peripheral elements reflexly inhibits liberation (Fig. 2, Table 2). If this is correct, the absolute amount of *B* in circulation is higher when μ is higher and lower when μ is lower. The interval ${}_wT_b$ ¹ (Fig. 3) involves a shift of μ from 1.0 to 4.0, ${}_wT_a$ and ${}_aT_b$ represents shifts from 1.0 to 2.5 and 2.5 to 4.0 respectively. If these changes are due to an increase in blood content of a single hormone, neither ${}_wT_a$ nor ${}_aT_b$ can be as great as ${}_wT_b$. The graph shows that ${}_wT_a$ is forty times greater than ${}_wT_b$. The single hormone hypothesis is therefore inadequate to explain the observed time relations. The two-hormone hypothesis implies that stimulation of peripheral elements reflexly excites the liberation of a second hormone *W* in quantity sufficient to override *B*. If this hypothesis is true, it is not surprising that ${}_bT_a$ and ${}_aT_b$ are relatively short because each involves only a *single* process of production or elimination of hormone. Similarly we should expect that ${}_aT_w$ and ${}_wT_a$ would be lengthy, because each involves *two competing* processes.

The existence of supernormal and subnormal phases in the time graph affords independent evidence of two antagonistic co-ordinators. The interval ${}_bT_w$ is con-

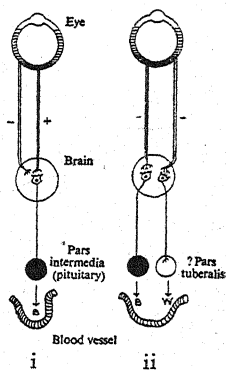


Fig. 2. Diagram to illustrate (i) one hormone and (ii) two hormone hypotheses of chromatic co-ordination in Amphibia. (Hogben & Slome, 1936.)

¹ Notation devised by Hogben. ${}_bT_w$ = time taken to pass from equilibrium on a black background to equilibrium on a white background. ${}_aT_b$ = time taken to change from equilibrium in complete darkness to equilibrium on a black background, etc.

spicuously greater than ${}_wT_b$. So if W exists, it accumulates in the circulation more slowly than it subsides. Comparison of ${}_aT_b$ and ${}_bT_w$ over equivalent distances of the melanophore scale implies that W is built up more slowly than B . Similarly

Table 2. *Reactions taking place during various time intervals according to the one-hormone and two-hormone hypotheses*

Time interval	One-hormone hypothesis	Two-hormone hypothesis
(1) ${}_wT_b$	Maximum increase of B due to release from reflex inhibition by peripheral retinal elements	Simple decrease of W while B remains constant. $B:W$ ratio increasing
(2) ${}_bT_w$	Maximum decrease of B due to reflex inhibition by peripheral retinal elements	Simple increase of W while B remains constant. $B:W$ ratio diminishing
(3) ${}_bT_a$	Decrease of B due to non-stimulation of basal retinal elements	Decrease of B due to non-stimulation of basal retinal elements
(4) ${}_aT_b$	Increase of B due to stimulation of basal retinal elements alone	Increase of B due to stimulation of basal retinal elements alone
(5) ${}_aT_w$	Submaximum decrease of B due to stimulation by basal retinal elements and prepotent reflex inhibition of B secretion through stimulation by peripheral retinal elements	Concomitant increase of B , and of W
(6) ${}_wT_a$	Submaximum increase of B due to simultaneous release from both (a) stimulation of B secretion by basal retinal elements and (b) prepotent reflex inhibition by peripheral retinal elements	Concomitant decrease of B , and of W

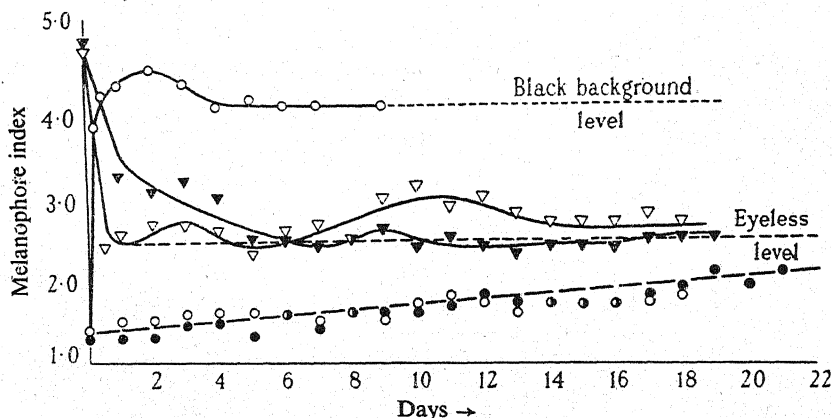


Fig. 3. Time relations of colour change in *Xenopus*. Circles of top curve represent transition from white to black background (dim light); triangles transition from black background to equilibrium in complete darkness. Circles of bottom curve represent transition from white background to darkness. Black circles and triangles represent animals from which eyes were removed at the beginning of the experiment (14°C). (Hogben & Slome, 1936.)

comparison of ${}_bT_a$ and ${}_wT_b$ implies that W subsides more slowly than B . The time graph of transition from darkness to a black background shows a pronounced supernormal curve (Fig. 4). This is readily accounted for in terms of two opposing co-ordinators W and B , of which the former is built up more slowly. It is unlikely

that localization of *B* and *W* retinal elements is so sharp that only the former and none of the latter is stimulated when the animal is transferred from darkness to a black background. So *B* is rapidly built up to a maximum. Later the small amount of *W* reaches its maximum and lowers the index. There is also a well-marked subnormal phase when animals equilibrated on a black background are transferred to complete darkness. This is susceptible of a similar explanation in terms of different subsidence rates of *B* and *W*.

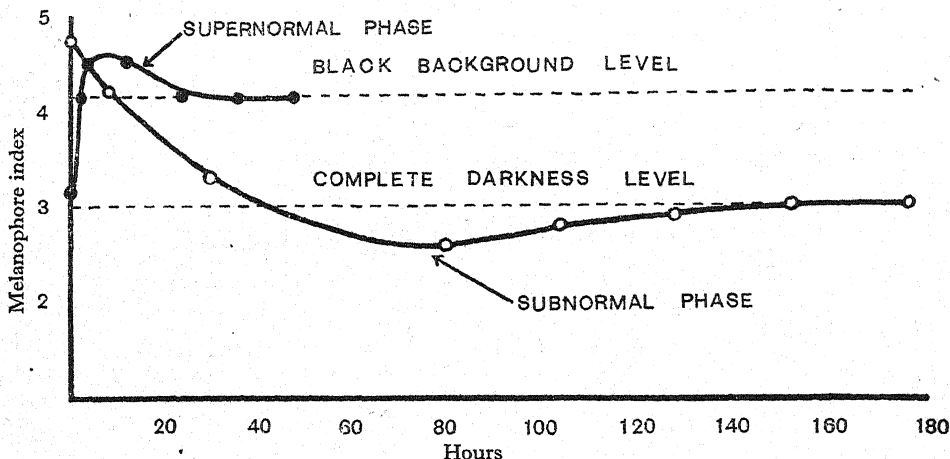


Fig. 4. *Xenopus*. Transition from intermediate melanophore condition in darkness to expanded condition on black background (●) and the reverse (○). 22° C. Redrawn from data in Hogben & Slome (1931).

(c) Table 3 shows the effects of partial and complete hypophysectomy in *Rana* and *Xenopus*. According to Hogben the significance of differences between responses of the two species is as follows: The pituitary of *Xenopus* is not of the anuran type.

Table 3. *Chromatic responses of normal and operated Rana and Xenopus*

In this and subsequent tables:

B = overhead illumination, black (non-reflecting) container.

W = overhead illumination, white (reflecting) container.

D = complete darkness.

μ = melanophore index in round figures (primary response ignored).

	<i>Rana</i> (Hogben, 1924; Waring & Clark, unpublished)			<i>Xenopus</i> (Hogben & Slome, 1931, 1936)		
	<i>B</i>	<i>W</i>	<i>D</i>	<i>B</i>	<i>W</i>	<i>D</i>
(1) Normal	$\mu = 5.0$	$\mu = 1.5$	$\mu = 3.0^*$	$\mu = 4.5$	$\mu = 1.5$	$\mu = 2.5^*$
(2) 'Whole' pituitary removed	$\mu = 1.0$	$\mu = 1.0$	$\mu = 1.0$	$\mu = 2.0$	$\mu = 2.0$	$\mu = 2.0$
(3) A.L.P. alone removed	$\mu = 5.0$	$\mu = 1.5$	$\mu = 3.0$	$\mu = 4.5$	$\mu = 1.5$	—
(4) A.L.P. and tuberalis removed	—	—	—	$\mu = 5.0$	$\mu = 5.0$	—
(5) P.L.P. alone removed	—	—	—	$\mu = 1.0$	$\mu = 1.0$	—

* Eyeless animals in light or darkness also equilibrate at this figure.

It is essentially that of a urodele. In *Rana* and *Bufo* the tuberalis is detached from the rest of the pituitary complex and is represented in adult life by two plaques that

lie far forward on the hypothalamus (Fig. 5). That of *Xenopus* and urodeles, as of larval Amphibia, is an undivided lip continuous with the anterior lobe. Removal of either the 'whole' pituitary or only the anterior lobe of *Rana* leaves the tuberalis *in situ*. Similar operations on *Xenopus* remove the tuberalis because it adheres closely to the anterior lobe. If the pars tuberalis or some organ under its control secretes a melanophore contracting hormone *W* antagonistic to the expanding hormone *B*, the differences between the two species brought out in items (2), (3) and (4) of Table 3 achieve a straightforward explanation.

Atwell (1941) has described two plaques on the hypothalamus of *Xenopus*. They are similar in structure and position to the pars tuberalis of *Rana*. He confirms the presence of a 'lip' at the front end of the anterior lobe. Atwell has not examined developmental stages. So the homology of the lip is uncertain. It is probably an unseparated part of the larval tuberalis.

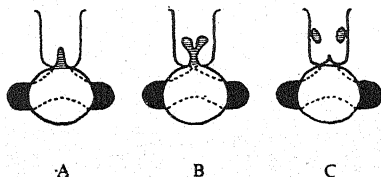


Fig. 5. Diagram to show anatomical relation of pituitary lobes in anura. A, adult *Xenopus*; B, *Bufo* at metamorphosis; C, adult *Rana*. Pars tuberalis: shaded. Pars intermedia: black. Pars anterior: white. (Hogben & Slome, 1936.)

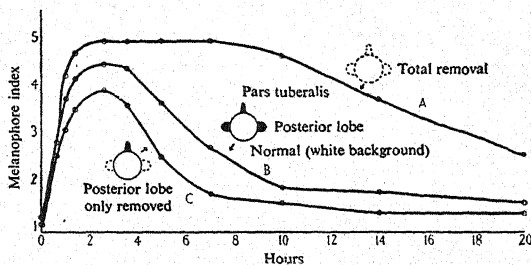


Fig. 6. Tolerance of three groups of *Xenopus* to *B*-containing extract. All animals the same weight and received equal injections. A, whole pituitary removed; B, intact animals; C, posterior lobe only removed. White background. Temp. 14° C. (Hogben & Slome, 1936.)

(d) Completely hypophysectomized animals show greatly decreased tolerance to injections of *B*. These results are inexplicable except on the assumption that the anterior lobe or its associated tuberalis antagonizes the injected *B*. After removal of the whole gland the *B* content in the circulation must be lower. So if *B* is the only contribution that the pituitary makes to chromatic co-ordination we should expect hypophysectomized animals to be more tolerant. The graph (Fig. 6) shows that they respond *more* readily to *B*-containing extracts.

No systematic investigations of elasmobranch chromatic responses were made until 1932. Lundstrom & Bard (1932) found that hypophysectomized dogfish are pale and are temporarily darkened by injection of posterior lobe extract. They made no observations on background response. Parker (1933) and Parker & Porter (1934)

described paling of *Raja erinacea* and *Mustelus canis* on a white background and darkening on a black background. Some of the new techniques introduced by Hogben & Slome were extended to elasmobranchs by Hogben (1936*a*) and Waring (1936*a*, 1938). The background response of all species is slow. Table 4 contains data comparable to that given for Amphibia in Table 3. Table 4 shows only one real difference between the behaviour of elasmobranchs and *Xenopus*. Total hypophysectomy evokes complete pallor of the former but not of the latter. In every other

Table 4. *Chromatic behaviour of normal and operated elasmobranchs*

	<i>B</i>	<i>W</i>	<i>D</i>
(1) Normal	$\mu = 5.0$	$\mu = 1.5$	$\mu = 3.0$
(2) Whole pituitary removed	$\mu = 1.0$	$\mu = 1.0$	—
(3) A.L.P. alone removed*	$\mu = 5.0$	$\mu = 5.0$	—
(4) P.L.P. alone removed	$\mu = 1.0$	$\mu = 1.0$	—

* Parker (1937) claimed that removal of the anterior lobe alone does not interfere with the normal responses of *Mustelus*. This is not confirmed by Abramowitz (1939). *Mustelus*, like other elasmobranchs, is permanently dark after removal of the anterior lobe alone.

respect responses of intact and hypophysectomized elasmobranchs are similar to those of *Xenopus*. Completely hypophysectomized *Scyllium*, like completely hypophysectomized *Xenopus*, is less tolerant to *B* than both intact animals and animals with the posterior lobe alone removed. So we concluded that elasmobranch melanophores are under bihumoral control.

The elasmobranch investigations showed that the new methods of Hogben & Slome could be applied to species other than *Xenopus*. They extended our knowledge of chromatic function to another vertebrate class. They did not provide any methodological advance. Observations on eels reinforced the arguments based on the responses of *Xenopus* and provided a new attack on the problem of the *W* substance.

Neill (1940) investigated responses of intact and blinded eels to various lighting conditions. Table 5 summarizes his data. The time relations are similar to those of

Table 5. *Responses of intact Anguilla*

Interval	Melanophore movement	Duration days	Interval	Melanophore movement	Duration days
${}_bT_w$	$5.0 \rightarrow 1.0$	20	${}_wT_b$	$1.0 \rightarrow 5.0$	20
${}_bT_d$	$5.0 \rightarrow 3.5$	2	${}_wT_d$	$1.0 \rightarrow 3.5$	27
${}_dT_w$	$3.5 \rightarrow 1.0$	22	${}_dT_b$	$3.5 \rightarrow 5.0$	9

Xenopus. ${}_bT_d$ and ${}_dT_b$ are short. ${}_wT_d$ ($\mu = 1.0 \rightarrow 3.5$) is greater than ${}_wT_b$ ($\mu = 1.0 \rightarrow 5.0$). ${}_dT_w$ ($\mu = 3.5 \rightarrow 1.0$) is greater than ${}_bT_w$ ($\mu = 5.0 \rightarrow 1.0$). Conclusions drawn from such data are outlined on p. 128.

Table 6 shows the responses of hypophysectomized eels. Completely hypophysectomized *Xenopus* are not so pale as those with the posterior lobe alone removed (Table 3). Hogben laid great stress on the significance of this observation. He

prophesied that if the two-hormone hypothesis was correct, an animal would be found in which the intermediate condition of the melanophores after hypophysectomy would be more strikingly demonstrated. *Anguilla* fulfils this requirement.

Hypophysectomized eels (*Anguilla vulgaris*), like hypophysectomized Amphibia and elasmobranchs, can be darkened temporarily by injection of *B* hormone. Completely hypophysectomized eels are less tolerant to *B* than intact ones. Except in one respect mentioned later, responses of intact eels to photic stimuli and responses evoked by operation and injection agree with those of *Xenopus* and *Scyllium*. For this reason it was concluded that eel melanophores are under bihumoral pituitary control. This conclusion is reinforced by evidence of a type not previously explored. The responses of intact *Xenopus* and *Anguilla* on transference to darkness from a

Table 6. Responses of hypophysectomized *Anguilla*

	<i>B</i>	<i>W</i>	<i>D</i>
(1) Normal	$\mu = 5.0$	$\mu = 1.0$	$\mu = 3.5^*$
(2) Whole pituitary removed	$\mu = 3.5$	$\mu = 2.0$	—*
(3) A.L.P. alone removed	$\mu = 5.0$	$\mu = 5.0$	—

* Hypophysectomized eyeless animals also equilibrate at about 3.5.

black background suggest that the hypothetical *W* substance is eliminated much more slowly than *B*. Waring (1940) recorded the effects of previous exposure to white and black backgrounds on the effects evoked by total hypophysectomy. Two groups of fish were used. One had been kept for 6 months on a black background. The other group was placed on a white background until the average melanophore index was 4.2. All animals were completely hypophysectomized and placed on a black background. The behaviour of the two groups is strikingly different (Fig. 7).

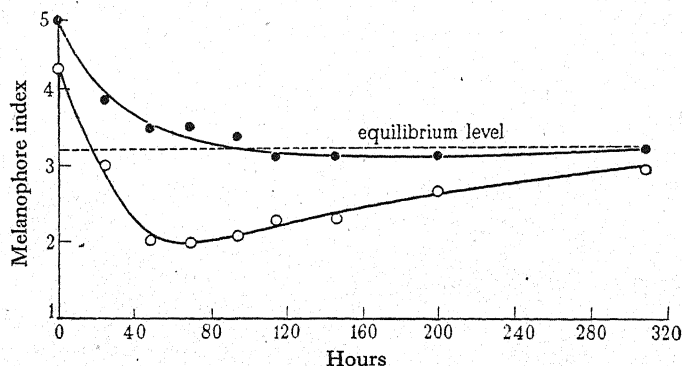


Fig. 7. Reaction of two groups of eels after total hypophysectomy. ● = previously equilibrated at $\mu = 5$. ○ = previously equilibrated at $\mu = 4.2$. Black background. (Waring, 1940.)

The second group pass through a subnormal phase before reaching equilibrium. This is intelligible in terms of the gradual subsidence of a melanophore contracting hormone.

The two-hormone hypothesis has not escaped criticism. (a) Parker (Parker,

1937; Parker & Scatterty, 1937) thinks the presence of W should be demonstrated by appropriate blood transfusions. It is difficult to perform critical experiments of this kind. Parker took blood from intact pale fish and Amphibia and injected it into dark ones. He got no response. Intact animals are not suitable for this class of experiment. According to the two-hormone hypothesis B is secreted under all conditions of overhead illumination. If the background is light reflecting, W is also secreted. Melanophores of intact animals on a white background rarely become fully contracted. So there is never an *effective excess* of W in circulation.

(b) Hogben's interpretation of the prolonged ${}_wT_d$ is given on p. 128. An alternative interpretation occurred to me. According to the one-hormone hypothesis stimulation of the peripheral retinal elements (white background) inhibits secretion of B . Inhibition may take place in one of two ways: (i) secretory elements inactivated, (ii) release of secretion prevented. If (i) is true, the gland of an animal equilibrated on a white background contains little B . Transition to darkness involves the *build up of B in the gland and circulation without retinal stimulation*. We should expect this to be slow. If (ii) is correct, the gland contains abundant B . Its release is prevented by a nervously co-ordinated mechanism. Transition to darkness involves *release of stored B into circulation*. We should expect this to be rapid. So we can furnish a plausible interpretation of ${}_wT_d$ in terms of one hormone if the glands of fish equilibrated on a white background contain little B . This can be subjected to direct test. The glands of pale animals on a white background contain abundant B (Waring *et al.* 1941).

(c) The first tolerance experiments were confined to intact and completely hypophysectomized toads. I suggested (Waring, 1935)¹ that the different tolerance of two classes of animals might be merely a measure of the effect of posterior lobe hormones on renal excretion of B . This objection was removed in the following year (Hogben & Slome, 1936) when data were published showing the differential tolerance of two classes of operated toads (Fig. 6). Both had the posterior lobe removed. One had the *pars glandularis in situ*.

(d) In a recent review of the co-ordinating mechanism of chromatic response of elasmobranchs Abramowitz (1939) drew attention to alternative interpretations of two classes of experiment described above (p. 131). He suggests that when the *pars glandularis* is removed there may be interference with the optico-hypophysial tract. If so, the loss of the white background response may be due to elimination of inhibiting impulses to the posterior lobe. The second criticism concerns the tolerance of normal and operated animals to injections of B . Abramowitz considers that the differential tolerance may be due to the lowered metabolic rate of animals without the anterior lobe.

The foregoing concerns the role of pituitary hormones in co-ordination. One other hormone has been invoked to explain certain responses. Adrenalin evokes melanophore contraction in many animals. Its effect on most reptilian and teleost melanophores is direct. Its effect on amphibian and elasmobranch melanophores is probably due to its vaso-constrictor action (Waring & Landgrebe, 1941).

¹ Oral communication to the Physiological Society.

Serious technical difficulties obstruct investigation of the role of adrenalin in normal chromatic behaviour. Adrenalin is secreted by chromaphil cells that are scattered in fishes and associated with cortical elements in land vertebrates. It seems impracticable to extirpate all the suprarenals of fishes. Removal of the chromaphil cells of land vertebrates necessitates removal of the cortical cells. This is fatal. There appears to be one solution to the problem applicable to reptiles, i.e. to remove the adrenals and maintain the animal with cortical extract. This has not been tried. The following experiments furnish suggestive evidence for the participation of adrenalin in pigmentary co-ordination.

(a) Faradic stimulation of the cord or palate region of normal or hypophysectomized catfish, *Ameiurus*, evokes contraction of *innervated* and *denervated* melanophores. Stimulation of posterior segments severed from the viscera evokes contraction of innervated but not denervated melanophores (Abramowitz, 1936b).

(b) Injection of serum from pale into dark *Phrynosoma* evokes local pallor (Redfield, 1918; Parker, 1938). Injection of Ringer has no effect. So it seems likely that there is a hormone that evokes melanophore contraction. In the small doses found to be effective by Parker (0.03 c.c. serum) the most likely excitant is adrenalin.

(c) Electrical stimulation of intact or hypophysectomized *Anolis* evokes uniform darkening of the post-orbital region and the appearance of clusters of black spots on the general body surface. This 'mottling' pattern is also shown by intact animals during emotional excitement. It does not occur in hypophysectomized epinephrectomized lizards on stimulation. It is evoked in such animals by the injection of adrenalin (Kleinholz, 1938a). This seems quite conclusive evidence for the participation of adrenalin in this type of response.

(3) *Peripheral nervous control superimposed on humoral control*

Elasmobranchs and Amphibia exhibit great uniformity of chromatic behaviour. Their responses are slow and in all of them there is pituitary control of melanophores. Such uniformity is not found within the groups Teleostei and Reptilia.

Chromatic response of most teleosts is fast. Ballowitz (1893) clearly demonstrated innervation of melanophores in the pike, *Esox*, as did Wyman (1924) in *Fundulus*. Early workers produced experimental evidence for nervous control of melanophores.

Pouchet (1876) cut the spinal cord in the mid-body region of the turbot. No effect was produced. Section of the trigeminal, destruction of the sympathetic nerves or section of the spinal cord below the point where it received the ramus communicans immediately caused the melanophores in the affected area to expand and lose their power of response. Lode (1890) cut the spinal cord of *Salmo* and applied electrical stimulation. Provided the sympathetic chain was left intact, all the melanophores contracted even when the cord was cut behind the region of stimulation. Von Frisch (1911) observed melanophore response after section and faradic stimulation of nerves of the minnow, *Phoxinus*. He found that (a) section of spinal nerves evokes expansion, (b) stimulation evokes contraction, (c) stimulation of the spinal cord or of the medulla produces contraction, (d) stimulation of the

fore-brain causes expansion. In the main his results have been confirmed on many species of fish (Wyman, 1924; Hewer, 1927; Giersberg, 1930; Fries, 1931; D. C. Smith, 1931*a*). Objections are raised to some of the early experiments because the circulation was left intact. This criticism does not apply to more recent experiments of Abramowitz (1936*a*) and of Neill (1940) using eviscerated fish.

Amphibian melanophore responses are co-ordinated by pituitary hormones. Peripheral nerves play no significant part. The most striking teleost responses are under direct nervous control. Early experiments with endocrines gave inconclusive results. So until about seven years ago it was customary to distinguish between forms with humoral and those with direct nervous control of melanophores.

Delayed appreciation of the significance of pituitary secretions in the regulation of teleost responses can be attributed to three causes:

(*a*) Injection of posterior lobe extract into intact pale animals on a white background evokes darkening in some forms (*Anguilla*, *Ameiurus*, *Phoxinus*), but not in others (*Fundulus*, perch).

(*b*) Immersion of isolated scales in pituitary solution gives conflicting results (summary in Abramowitz, 1936*b*).

(*c*) Hypophysectomy and quantitative study using the melanophore index were accomplished only recently on teleosts.

When it became apparent that the pituitary played a significant part in co-ordinating responses of some teleosts, phrases became common such as 'direct nerve control supplemented by a humoral influence'. Since it was evident that hormones were more important in some species than in others, various *ad hoc* hypotheses resulted. No attempt was made to define the exact role of pituitary secretions and peripheral nerves. So it was impossible to formulate a general evolutionary hypothesis that would take account of published data from various species.

The first attempt to formulate a general hypothesis which defined the precise role of humoral and nervous agencies is due to Hogben. It was the direct result of intensive study of time relations. Hogben & Landgrebe (1940) made a detailed study of the chromatic responses of *Gasterosteus*. This investigation shed light on the interplay of humoral and nervous control where both are significant agents in chromatic response. Their time graphs exhibit two noteworthy features:

(*a*) Initial stages of background reversal are rapid and consistent with direct nervous control of melanophores. The final stages are prolonged and consistent with humoral action.

(*b*) There is a protracted transition to equilibrium in darkness. This is of a different order of magnitude from the background response. Its time relations are more like those of Amphibia and as such are more consistent with humoral control.

The results of this investigation suggest that direct nervous control has been superimposed on the more archaic pituitary mechanism. The separate role of the two can be analysed only by distinguishing between two essentially different aspects of colour change:

(a) What happens when an animal is brought out of darkness into light and vice versa.

(b) What happens when an animal is transferred from overhead illumination in a white tank to overhead illumination in a black container and vice versa.

As a result of their findings Hogben & Landgrebe put forward the following hypothesis: In all vertebrates what happens when an animal is transferred from an illuminated black tank to darkness and vice versa results from secretion or excretion of the *B* hormone of the pars intermedia. What happens when an animal is transferred from an illuminated black to white tank and vice versa results either from the secretion or excretion of a second pituitary hormone¹ *W* or from direct nervous control secondarily superimposed upon, and to a greater or less extent replacing, a more archaic humoral mechanism.

One implication of this hypothesis is that hypophysectomized animals on a black background should equilibrate at a melanophore index not higher than that of intact animals in darkness. This is true for *Anguilla*. It is probably true of *Ameiurus*. These are the only two teleosts for which melanophore index readings or melanophore measurements have been made after hypophysectomy.

Observations on *Fundulus* provide independent confirmation that nervous agencies have been superimposed upon an archaic humoral mechanism. Initial stages of background response in this fish are rapid and undoubtedly result from direct peripheral nervous action. Final changes in attaining equilibrium are less rapid (Table 7). From considerations set out above we should expect that these final changes are co-ordinated by humoral agencies. Abramowitz (1937) did in fact find that serum from pale fish on a white background contains less melanophore expanding potency than serum from dark fish on a black background.

The negative results of injecting pituitary extracts into some species (e.g. *Fundulus*) in the pale state induced by illumination on a white background have no relevance to the validity of Hogben's hypothesis. Its universal validity would be disproved if pituitary extract produced no effect on the state of pallor which supervenes *when melanophores are freed from nervous control*. Contracted melanophores of *Fundulus* freed from nervous control are found in two circumstances: (a) intact animals in complete darkness, (b) animals with nerves severed and kept on a white background for long periods. No experiments have been made on (a). Kleinholz (1935) and Abramowitz (1937) both found that denervated melanophores react to pituitary injections.

The separate role of pituitary hormones and peripheral nerves can be dissected by the following series of experiments: (a) Accurate observations of the responses of intact and hypophysectomized animals to (i) background reversal, (ii) transition to darkness from illuminated backgrounds and *vice versa*. (b) Effect of pituitary extracts on (i) intact pale fish on a white background, (ii) intact pale fish in complete darkness, (iii) denervated melanophores. (c) Estimation of the effective *B* content of serum from fish kept under different conditions.

¹ Or in terms of the one-hormone hypothesis (Table 2) an absolute reduction or increase of *B*. According to either hypothesis there is a reduction of effective *B* in circulation when an animal is transferred from an illuminated black to white tank.

For no one teleost have all these tests been made. American workers (Matthews, 1933; Parker, 1936*a*; Desmond, 1924; Abramowitz, 1937; Osborne, 1938) have shown that *Ameiurus* and *Fundulus* survive hypophysectomy. They have estimated the *B* content of serum and the effect of pituitary injections on intact pale fish and fish with denervated melanophores. Their observations show that both pituitary hormones and peripheral innervation are significant agencies in co-ordination. In neither *Ameiurus* nor *Fundulus* have accurate observations been made on the behaviour of animals transferred from white to black backgrounds and the reverse. What is more important, no detailed observations have been made on the behaviour of animals brought from complete darkness to either white or black backgrounds. So it is impossible to form clear ideas of the separate contribution of pituitary and nervous agencies.

Hogben & Landgrebe (1940) and Hogben & Clark (unpublished) have analysed the time relations of *Gasterosteus* and *Phoxinus*. Their findings provide suggestive evidence for the separate contributions of humoral and nervous co-ordinators. Little work has been done on the effect of hypophysectomy on these fish. So the following series of types illustrating increasing dominance of direct innervation is tentative:

(i) *Anguilla*. Chromatic response of intact eels is slow. Hypophysectomized animals on a black background equilibrate at approximately the same melanophore index ($\mu = 3.5$) as intact animals in complete darkness. Unlike Amphibia and elasmobranchs completely hypophysectomized eels exhibit a limited background response. This is fairly rapid and is nervously co-ordinated. Direct nervous control plays no part in the responses of intact animals. Injection of pituitary extract darkens intact pale fish on a white background (Waring, 1940).

Table 7. *Teleost background reversal times*

Fish	${}_wT_b$	${}_bT_w$	Reference
<i>Anguilla</i>	20 days	20 days	Neill (1940)
Pleuronectids*	2-7 days	2-7 days	Osborne (1939)
(various spp.)			
<i>Ameiurus</i> *	1 hr.	3 hr.	Abramowitz (1936 <i>b</i>)
<i>Salmo</i>	10 hr.	30 min.	Neill (1940)
<i>Phoxinus</i>	60 min.	60 min.	Hogben & Clark (unpub.)
<i>Gasterosteus</i>	60 min.	60 min.	Hogben & Landgrebe (1940)
<i>Fundulus</i> *	Large change in 1 min., complete change in ap- prox. 2 hr.	Large change in 2 min., complete change in ap- prox. 4 hr.	Parker (1936 <i>a</i>)
<i>Lebistes</i>	35 min.	7 min.	Neill (1940)

* Based on macroscopic observations. Time relations estimated by macroscopic indices are apparently more rapid than those based on the melanophore index.

(ii) *Ameiurus*. Background responses are more rapid. Hypophysectomized animals on a black background equilibrate at an intermediate melanophore index. Hypophysectomized animals show a limited background response. Nervous control is significant in background responses. Injection of pituitary extract darkens intact pale fish on a white background (Abramowitz, 1936*b*; Parker, 1936*a*; Osborne, 1938).

(iii) *Phoxinus*. Background responses are rapid. Nervous agencies dominate the background response. Time graphs show that the final stages of the background response are prolonged and consistent with humoral action (Hogben & Clark, unpublished). Infundin darkens intact pale fish on a white background (Abolin, 1925).

(iv) *Gasterosteus*. Time relations of response to background reversal and transition to darkness are almost identical with those of *Phoxinus*. According to Osterhage (1932) pituitary extract darkens pale fish on a white background. Jores (1936) claims that it has no effect.

(v) *Fundulus*. Response to background reversal is rapid. Nervous agencies dominate the background response. Hypophysectomized fish show background responses, but 'never become as dark as normal animals when exposed for equal periods to a black environment' (Abramowitz, 1937). Pituitary extracts do not darken intact pale fish on a white background (Kleinholz, 1935; Abramowitz, 1937).

Background reversal times (Table 7) provide suggestive evidence for different degrees of peripheral nervous control in other species.

In some reptiles (cf. p. 127) humoral control seems to have been almost entirely superseded. Reptile pigmentary responses are not susceptible of quantitative treatment based on the melanophore index. The time of transition from pallor in darkness to dark skin on a black background, is too short for the build up of hormones to an effective level in the circulation. There is conclusive evidence for direct nervous control. So Zoond (Sand) & Eyre (1934) and Sand (1935) have formulated a scheme which invokes only nervous agencies. For two reasons we cannot be certain that hormones play no part. Sand did not record observations on background reversal. His time graphs concern only transition from darkness to illuminated backgrounds. He did not deal with the effects of hypophysectomy.

(4) *Double innervation hypothesis*

Bert (1875) first proposed the theory that melanophores receive a double innervation of sympathetic and parasympathetic fibres, which control their contraction and expansion respectively. This theory has been strongly supported by Parker and his school. Two classes of evidence have been invoked in its support: (i) effect of drugs, (ii) behaviour of denervated melanophores.

Injection of drugs has been studied by Hogben & Winton (1922*b*), Wyman (1924), Giersberg (1930-2), D. C. Smith (1931*b*) and others. Hogben and Winton found that frog melanophores are relatively insensitive both to sympathomimetic and to parasympathomimetic drugs. Smith's results on fishes can be tabulated as follows:

- (a) Cocaine, a sympathetic 'stimulant', contracts melanophores.
- (b) Ergot, a sympathetic 'depressant', inhibits this action.
- (c) Pilocarpine and physostigmine, parasympathetic 'stimulants', cause expansion.
- (d) Atropine, a parasympathetic 'depressant', inhibits this.

Spaeth & Barbour (1917) claimed that ergot reversed the effect of adrenalin on melanophores (of detached scales) as it reverses the action of adrenalin on the uterus. Recently Vilter (1937) claimed that elasmobranch melanophores are innervated. He

based his conclusion partly on the injection of atropine. The results of such experiments would be convincing evidence for sympathetic or parasympathetic innervation of melanophores, if sympathomimetic and parasympathomimetic drugs consistently produced the effects of sympathetic or parasympathetic stimulation. Since there is no absolute correspondence between the action of a drug and the innervation of an effector organ, the action of any single drug cannot tell us whether the chromatophore is innervated by the sympathetic or parasympathetic—nor even whether the melanophores are innervated at all. The truth is that when a close correspondence between the action of a drug and the influence of nerves has been shown to exist, the relevant evidence is drawn from a single class of effector organs (plain muscle) and the known descriptions between the relation of sympathomimetic and parasympathomimetic drugs and plain muscle does not apply to at least one other effector organ which has been closely studied, namely glandular epithelium. Therefore it is necessary to emphasize two points: (1) In the words of Langley (1921) the action of drugs 'depends rather on the nature of the tissue than on the nervous system supplying it'. (2) Analogous, though less striking, effects have been recorded of Amphibia, though the bulk of evidence indicates that melanophores are not under direct nervous control.

The only cogent case that can be made for the use of drugs in research on chromatic behaviour is that they help us to get some idea of effector time (Osterhage, 1932; Hogben & Landgrebe, 1940; Waring & Landgrebe, 1941). Drug reactions do not provide sufficient reason for believing in double innervation of melanophores.

A second class of evidence invoked in support of double innervation concerns the behaviour of denervated melanophores. When fin rays of a pale teleost are cut, a dark band appears distal to the cut. When the nerves are electrically stimulated the melanophores contract. Pouchet (1876) and von Frisch (1911) regarded the melanophores in denervated regions as unexcited because they were separated from impulses issuing from the central nervous system. This view has been generally accepted. Parker has made observations which make it unlikely that this interpretation is entirely correct. A cold block placed between the cut and the periphery of the rays evokes melanophore contraction in the denervated region. The melanophores expand again when the block is removed. Melanophores in a denervated region eventually contract if the fish is in a white container. They expand again if a new cut is made between the original cut and the periphery before complete degeneration of the peripheral fibres and before new fibres have grown.¹ Parker interprets these facts in the following way: (1) Melanophores have a double innervation of 'contracting' and 'expanding' fibres (Mills, 1932*a, b*; Parker, 1934*a*). (2) Cutting the nerves sets up a sustained injury discharge in the expanding, but not in the contracting fibres (Parker, 1934*b*). (3) Electrical stimulation of peripheral nerves excites the contracting, but not the expanding fibres (Parker, 1934*b*).

¹ The relevant details are: Dark denervated bands of *Fundulus*, if maintained on illuminated white container, fade in 2-9 days (Parker & Porter, 1933; Abramowitz, 1935, 1937; Kleinholz, 1935). Degeneration of the distal fibres is complete on the 5th day (Parker & Porter, 1933). Regeneration of nerves begins 18-20 days after cutting (Parker & Porter, 1933; Abramowitz, 1935). All estimations based solely on melanophore activity.

If we make these three suppositions we certainly attain a consistent explanation of the phenomena concerned. According to this hypothesis, local application of cold blocks the impulses in fibres whose stimulation evokes melanophore expansions. New cuts set up new injury discharges in the as yet undegenerated fibres. There is no inherent unlikelihood of the existence of such injury discharges. The fact that persistent injury discharges have not yet been demonstrated in the peripheral nerves of cold-blooded vertebrates makes it highly desirable that experiments should be made to *measure* these impulses in teleost material. Meanwhile a simpler interpretation has not been disproved. It is based on the hypothesis advocated on p. 137. When *Fundulus* is transferred from black to white background two things happen. (a) Peripheral nerves transmit impulses that *override* the influence of *B* and evoke melanophore contraction. This is rapid. (b) There is a slow reduction in *B* content of the blood. Nerve section on a white background frees melanophores from nervous control. If process (b) is not complete, melanophores expand in response to *B* in circulation.

One class of experiment would discriminate between these two interpretations. If the second is true, the darkening described by Parker should not occur in hypophysectomized fish unless such darkening is due to a local vasomotor disturbance. According to Osborne (1938) Abramowitz found that 'denervated bands (of *Fundulus*) did not darken in animals which had been hypophysectomized several days'.

Study of melanophores in denervated regions furnished other data that has been interpreted as supporting the double innervation hypothesis. Mills (1932*a*) denervated caudal areas in *Fundulus*. When pallor of these areas had been induced by sojourn in a white tank, the fish were transferred to a black one. The innervated melanophores expanded as did some of the denervated ones on the fringe of the band. On transfer to a white background some of the latter did not contract. Thus some fringe melanophores can expand fully but not contract fully. Similarly some can contract but cannot expand. Abramowitz (1935, 1936*a*) observed that new fibres grow out from cut stumps to their effectors (melanophores). He studied those melanophores which respectively contracted during white background adaptation or expanded during black background adaptation in a denervated caudal area after lapse of sufficient time for regeneration of nerves. He concluded that melanophores may regain power to expand without regaining power to contract or vice versa. This is the best evidence so far produced for double innervation. It is not conclusive.

Parker considers that the double innervation hypothesis rests on satisfactory evidence. He states that the melanophores of both *Fundulus* and *Ameiurus* receive a double innervation of 'dispersing' and 'concentrating' fibres. Starting from this premise he concludes that in *Mustelus*, *Squalus* (*Aconthias*), and *Phrynosoma* there are only 'concentrating' fibres. A chisel cut in dark fins of *Mustelus* and *Squalus* evokes a pale band distal to the cut. In *Mustelus* the same effect can be obtained by faradic stimulation. Cuts in pale fins do not produce a dark band (summary in Parker, 1937). Parker believes that such experiments show that normal pallor is due to direct peripheral action of 'concentrating' fibres. Concerning the observed

facts of pallor produced in this way there can be no doubt. Several interpretations of them are feasible. Abramowitz (1939) was unable to produce similar results by severing individual nerves. His experiments seem to show that the pale area is due to direct or indirect interference with the circulation. If it could be shown that the melanophores are directly innervated, or even that the effects are due to severing vaso-motor nerves, we should still have to satisfy ourselves that normal pallor in the transition from black to white backgrounds occurs with a rapidity comparable to the effects of stimulating or severing nerves. That Parker has had this difficulty in reconciling his interpretation of fin cutting experiments with his own direct observations on the time-relations of normal responses is shown by the following quotations from one and the same publication (1937): (a) 'Sluggishness reflects the general character of a system built on the basis of water soluble neurohumors... as contrasted with one which is more predominantly nervous' (p. 243). (b) The paling reaction in *Mustelus* 'requires some two days' (p. 226). (c) Melanophore contraction in *Mustelus* 'results from direct nervous action' (p. 236).

Parker's (1938) evidence for direct nervous control of *Phrynosoma* melanophores can be summarized as follows: (a) Electrical stimulation of the mouth or cloaca evokes reversible pallor. (b) After breaking the cord stimulation of the mouth evokes pallor only in the anterior half of the body. (c) Electrical stimulation of three or four adjacent spinal nerves evokes pallor in the innervated region. (d) Preparations denervated on one side and stimulated on the floor of the mouth pale everywhere except in the denervated portion. (e) Stimulation of the femoral nerves of decapitate preparation evokes pallor in that leg.

Parker summarizes his conclusions from these data as follows: 'Since general blanching may be excited reflexly by faradic stimulation of the mouth and local blanching by stimulating nerve trunks, and since both these types of blanching may be locally blocked by nerve cutting, I conclude that *Phrynosoma* possesses concentrating melanophore nerve fibres which are directly concerned with its pale state.'

With the exception of (e) all these experiments are the same in principle. They involve the stimulation of nerves with the blood system still intact; and the limitations of this method have been repeatedly emphasized. Since most mixed nerves contain vasomotor fibres, pallor in such experiments may be brought about by either vasoconstriction or by stimulating melanophore nerves. In short, they cannot provide unequivocal evidence (p. 125). Kleinholz (1938*b*) obtained reversible pallor in *Anolis* after faradic stimulation of *intact* animals. The melanophores of this reptile are not directly innervated. We need evidence from eviscerated *Phrynosoma*, and it is not clear that the relevant condition was fulfilled in the last experiment. We are not told whether decapitation involved cessation of blood flow. It may or may not be significant that Parker records pallor after stimulation of the decapitated animal, but not reversible pallor. Redfield (1918) described experiments of a similar type and drew similar conclusions. Parker found that severing nerves to pale limbs had no chromatic effect. He interprets this by assuming that *Phrynosoma* has no nerve fibres which promote expansion. If we had data with reference to background reversal after nerve section, we should know whether direct innervation does or does

not play a significant role in the co-ordination of chromatic response in this animal. No recorded experiments on *Phrynosoma* provide conclusive evidence for believing that the melanophores of this animal are under direct nervous control. No recorded experiments prove the absence of such control.

There is no intrinsic unlikelihood of double innervation of melanophores. The evidence for its existence is insufficient. Some objections raised on p. 141 could be settled by one carefully conducted experiment. In none of the three species for which single innervation of the melanophores has been advocated by Parker is there acceptable evidence for any peripheral nervous intervention in the responses of the intact animals.

(5) Neurohumoral hypothesis

Co-ordination may be humoral (hormonal), e.g. excitation of the vertebrate pancreas by liberation of secretin into circulation, or nervous, e.g. the reflex activity of skeletal muscle.

According to customary usage *hormone* denotes a substance that is liberated into the blood stream and exerts its influence over remote effector organs. In nervous co-ordination impulses are conveyed direct to effector organs by nerve fibres. Concerning the nature of the excitatory process at the nerve effector junction two views are generally expressed. One is that there is a simple conduction of the same type of physico-chemical disturbance that constitutes the impulse in the nerve. Alternatively, the terminal arborization of the nerve may act in a manner analogous to a gland, producing a drug-like substance that excites the neighbouring effector or neurone. Various names have been suggested for such excitants, e.g. neurohumor (Fredericq, 1927; Parker, 1932), transmitter (Dale, 1934) and neurohormone (Huxley, 1935). The use of the word *neurohumor* for substances effective between nerve-effector junctions of microscopical dimensions is open to no reasonable criticism; but it cannot be too strongly emphasized that *neurohumor* so defined has little in common with *hormone* as proposed by Bayliss and Starling and subsequently used in the literature of endocrinology.

Parker and his collaborators have invoked a neurohumoral hypothesis theory to explain certain phenomena of chromatic response. Parker's writings on neurohumoralism fall into two quite distinct categories: (a) Experiments on teleost species (e.g. *Fundulus*) in which there is undoubtedly 'direct' nervous control of melanophores. It is claimed that the nerve endings in close proximity to the melanophores influence their effector by liberation of neurohumors. (b) Arguments to show that there is no valid distinction between humoral and nervous co-ordination. These will be discussed separately.

(a) Parker based his neurohumoral interpretation of melanophore control in *Fundulus* and *Ameiurus* on the following considerations (cf. p. 140):

(1) In pale fish in which dark bands have been induced, the expanded melanophores are believed to be under the influence of persistent injury currents. They are not paralysed, as is generally assumed.

(2) When fish are kept on a white ground the dark melanophore band gradually

pales owing to contraction of melanophores. There is a definite gradient. Melanophores at the side limits of the band contract first.

(3) When a once darkened band has paled and the fish is transferred to a black tank, the denervated band gradually darkens, melanophore expansion being progressive from the outside of the band.

Parker contends that the gradual elimination of pale or dark denervated bands can be explained only by the diffusion of oil-soluble neurohumors from adjoining innervated areas. Sand (1937) implies that such effects are difficult to explain in any other way. It seems to me that these results will only be difficult to explain otherwise if it can be demonstrated that they occur in hypophysectomized fish. Abramowitz (1937) put pale hypophysectomized fish with denervated bands into black tanks. In my opinion his results strongly suggest that expansion and contraction of melanophores in denervated bands is due to fluctuation of pituitary substances in circulation. Hypophysectomized *Fundulus* were transferred from an illuminated white to black container. The melanophores in the denervated bands of five fish remained fully contracted. In the remaining six fish they expanded slightly. Abramowitz makes no mention of the gradient upon which Parker lays such emphasis. Parker (1936a) has figured the gradual elimination of pale bands in hypophysectomized *Ameiurus*, but Osborne (1938) thinks that Parker's observations were made too soon after operation to give reliable data.

With regard to blood-borne hormones (i.e. hormones *sensu stricto*) that induce pallor, D. C. Smith (1931b—*Phoxinus*) and Abramowitz (1936b—*Ameiurus*) have described such a substance, which is not of pituitary origin. It is suspected to be adrenalin. Parker has claimed that this substance cannot be instrumental in the changes under discussion because injection of adrenalin into pale fish with a newly induced dark band causes rapid disappearance of the dark colour without formation of a gradient. This argument is of doubtful significance, unless the dose (unstated) is minute, because of the extreme rapidity with which this drug affects teleost melanophores.

Parker has also formulated a neurohumoral interpretation of chromatic response for elasmobranchs. In *Fundulus* and *Ameiurus* there is good evidence for direct nervous control of melanophores. The only relevant issue is whether nerves activate melanophores by the mediation of lipohumors and whether the observed results of nerve cutting can be explained by their activities. Among elasmobranchs there are only two species—*Acanthias* and *Mustelus*—for which there is any experimental evidence suggesting direct, nervous control (p. 141). Most of Parker's recent work has been done on *Mustelus*. He does not deny that all elasmobranchs darken under the influence of the neuro-intermediate hormone 'B'. Cuts made transverse to the fin rays elicit pale areas in the dark fins of *Acanthias* and *Mustelus*. The possible interpretations of these facts have been discussed by Parker (1936a), Waring (1938) and Abramowitz (1939). Parker assumes that the production of pallor indicates direct innervation of the melanophores. He interprets the gradual obliteration of the pale zone from the side as being due to the gradual diminution of a lipohumor and this allows the pituitary hormone 'B' to exert its effect. Parker (1935c) extracted

pale fins with olive oil (or ether) and on injecting it under the skin of a dark fish obtained a pale area. Control injections of oil and of sea water extracts of pale skins had no effect. From these observations he claims that he has extracted a lipohumor which is liberated by nerves whose direct effect on the melanophores is to induce contraction. Two comments may be made on this. (i) It is not generally agreed that peripheral nervous action is responsible for normal pallor in *Mustelus* (p. 142). (ii) If the existence of such direct nervous control is proved, it will still be necessary to show that the oil-soluble excitant is produced from nerve endings and affects the melanophores directly.

(b) All workers agree that visual control of chromatic response initially involves discharge of nervous impulses to the brain. In forms with humoral control (e.g. frog), impulses from the brain evoke release of pituitary hormones. So if the word neurohumoral had not been used more appropriately by Parker and others to describe another physiological event, it would be a fitting description for a sequence that involves reflex stimulation of the pituitary or other endocrine.

In forms with peripheral nervous control (e.g. *Fundulus*) impulses pass by nervous tracts to within microscopic distances from the melanophores. They then activate the effector by release of neurohumors or by other means. Parker insists that there is no valid distinction between the release of pituitary secretions into the blood stream and the release of excitants from nerve terminals in close proximity to effectors.

If melanophores have nerve endings which influence them by liberating drug-like substances, the fact is of considerable physiological interest and Parker's work on denervated areas of *Fundulus* is a contribution to this. It is, however, a purely verbal compromise between issues which are not factually consistent to claim that in the light of the neurohumoral hypothesis 'the distinction of a nervous and humoral excitation really vanishes' (Parker, 1937). Co-ordination by a blood-circulated hormone has different physiological attributes from nervous co-ordination. Whether nerves excite their effectors by the liberation of drug-like substances or by the polarizing action of ions at the separating membranes does not affect the issue.

The apparent universality implied by the neurohumoral hypothesis as put forward by Parker has its roots in the unsatisfactory state of present nomenclature. There would be no controversy about this issue if biological writers (a) used Schaefer's word *autacoid* for substances with specific excitatory properties but no proved physiological role and restricted the use of the word *hormone* in conformity with the definition of Bayliss and Starling to such substances as the B component of pituitary secretion; (b) limited the term *neurohumor* to drug-like substances released from nerve terminals which excite effector in close proximity. If Parker had relied on these definitions, no confusion would have arisen. No clarification of the ambiguity that results from the use of the same word in two different senses is gained by redefining neurohumors as 'certain substances as possible activators of colour cells and in no necessary way coupled with the sources of these substances' (Parker, 1938).

(6) *Evolution of melanophore control in vertebrates*

Comparison of the geological age of different taxonomic groups (Zittel, 1938; Berg, 1940) that exhibit respectively humoral and predominantly nervous co-ordination of *background responses* (Fig. 8)¹ provides suggestive evidence for a historically later participation of peripheral nervous control in fishes (cf. p. 137). The number of reptile species investigated is too small to justify even tentative generalisation. There is pituitary control of melanophores in *Anolis* and direct

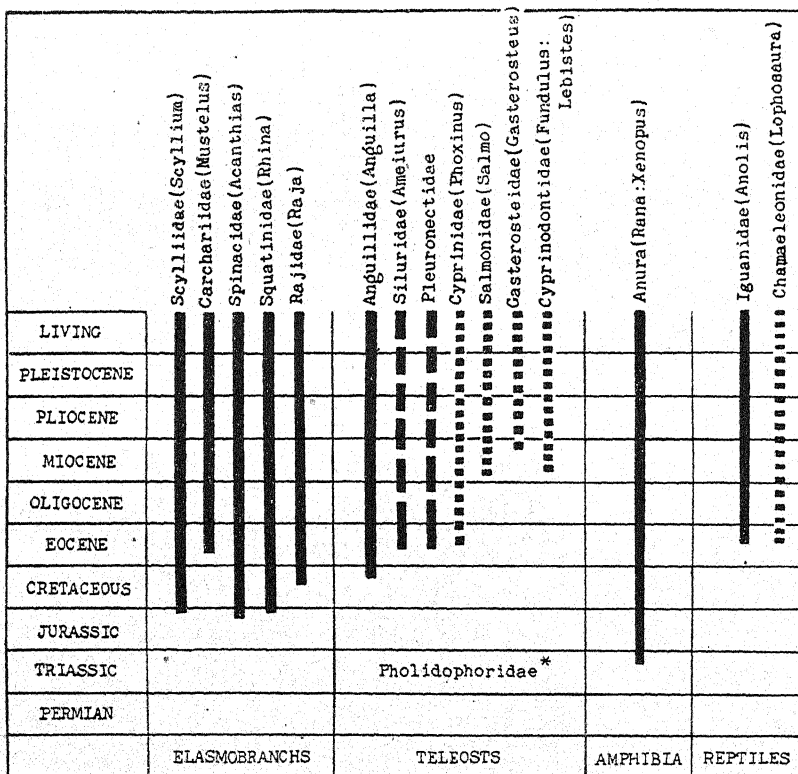


Fig. 8. The geological age of vertebrate groups with different mechanisms for co-ordinating melanophore background responses. — predominantly humoral. - - - mixed humoral and nervous. predominantly nervous.

* 'Seem to be directly ancestral to modern teleosts' (Zittel, 1938).

nervous control in *Lophosaura*. The chromatic behaviour of *Lophosaura* exhibits distinctive features which may indicate that it has evolved along lines different from those envisaged for teleosts. No reptile has been described which appears to bridge the gulf between *Anolis* and *Lophosaura*. *Phrynosoma* (Iguanidae) is claimed (Parker, 1938) to have mixed humoral and nervous control. The evidence for pituitary control is good: evidence for direct nervous intervention is not convincing.

¹ In the construction of this table (Fig. 8) I have had the advantage of discussion with Dr Westoll.

If rapid background response of melanophores has selection value, then it follows that both rapid co-ordination and a more rapid effector time must be developed together. This has happened in forms such as *Lebistes*, *Gasterosteus* and *Salmo* where the effector speed is of the order of 3 min. No case is recorded where a slow effector time is associated with direct innervation. In *Anguilla* any advantage that might accrue from the increased effector speed is cancelled by the ineffectiveness of the direct innervation. This enforces evidence advanced elsewhere to show that the eel occupies a transitional stage in the evolution of chromatic co-ordination. Although there is direct innervation of the melanophores of the eel, nervous control of background pallor has not yet superseded the more archaic humoral control. The relation of the co-ordinating system to the effector suggests that the same selective process promoted a more rapid effector reaction time concurrently with greater participation of the peripheral sympathetic innervation, and that the initial evolution of the latter was due to agencies which had no direct relation to the chromatic function.

III. SUMMARY

1. Two classes of melanophore responses to light can be distinguished:
 - (a) A primary, direct or non-visual response involving the expansion of the melanophores in light and their contraction in darkness. This non-visual response may be a co-ordinated response or the melanophore may be an independent effector.
 - (b) A secondary or visual response when the melanophores contract if the visual field is coincident with a light-scattering field and expand if the animal is exposed to superior illumination in surroundings which do not reflect or scatter light coming from above.
2. Co-ordination of melanophore responses in elasmobranchs and Amphibia is by means of pituitary hormones circulating in the blood.
3. A direct nervous control of melanophores has been superimposed on the more archaic humoral mechanism to a varying extent in different genera of teleosts. Sufficient is now known of this group to formulate tentatively a general scheme applicable to all forms, according to which the two components—humoral and nervous—are developed to a greater or less extent in various genera.
4. Co-ordination of melanophore responses in some reptiles is solely humoral. In others only nervous agencies have been discovered. Insufficient data are available to construct a scheme showing the phyletic relationship of the two systems between the members of the group.
5. The kind of evidence that is acceptable for the existence of the various co-ordinating systems is discussed.
6. The merits of the bihumoral, double innervation and neuro-humoral hypotheses are discussed.
7. An evolutionary scheme is appended which may tentatively be taken to indicate that direct nervous control is phylogenetically a later development than humoral control.

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* Review.

INSECT SURVIVAL IN RELATION TO THE RATE OF WATER LOSS

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I. SATURATION DEFICIENCY AS A MEASURE OF ATMOSPHERIC HUMIDITY FOR ENTOMOLOGISTS

THE importance of atmospheric moisture to insects was recognized long ago. Attention was first paid to the *wetness* of the air which was expressed in the combined terms of relative humidity and temperature. But since fasting insects tend to dry up, it eventually seemed better to think of humidity in terms of *drying power* of the air, i.e. as its saturation deficiency. This view was first put before entomologists in 1924 by Bacot & Martin; they knew that plague epidemics and saturation deficiency were correlated, and they set out 'to ascertain the separate influences of temperature and drying upon the longevity of fleas with a view to the interpretation of the epidemiological facts'.

Buxton and others tried to find if it was possible, by using saturation deficiency with insects, 'to speak of the combined effect of temperature and humidity... irrespective of differences of temperature within considerable limits' (Buxton, 1931). In those early days, although there were 'no actual measurements of water lost from the surface of the insect, under controlled atmospheric conditions... several authors have exposed insects to a range of combinations of temperature and humidity and have recorded either the conditions which are fatal in a definite period, or else the length of exposure which the insect is capable of surviving. From a consideration of the work of these authors, it is apparent that one of the causes of death is loss of water. It is therefore possible to take the facts accumulated in these

papers and to use them to test the hypothesis that loss of water is determined by saturation deficiency' (Buxton, 1931).

Thus the importance of the drying power of the air was emphasized, although with no insects had it been shown that death was due *only* to water loss or that if no other factors intervened water loss remained the master factor in survival. This fact casts doubt on the last statement in the above quotation (see Mellanby, 1935).

Eventually direct experiments on water loss from insects were made; but there is to-day still no general and simple law relating either survival or water loss to saturation deficiency, although they seem to be more closely correlated with this than with any other measure of humidity.

II. HYPOTHETICAL LONGEVITY-SATURATION DEFICIENCY CURVES IF THE RATE OF WATER LOSS IS LINEARLY RELATED TO SATURATION DEFICIENCY AND LONGEVITY IS LIMITED BY WATER CONTENT

(1) *Note on terminology*

In the literature on insects and saturation deficiency there is a confusion of the terms used to describe direct and inverse proportion. Definitions are, therefore, necessary.

Direct proportion. x may increase as y increases in many different ways: but x is said to be *directly proportional to* or to *vary directly as* y only when x/y is constant, or

$$x = Ky. \quad (1)$$

The graph of x against y is then linear and passes through the origin (Fig. 1a).

Such terms as *proportional to* or *varies as* do not qualify the type of variation and cannot be used for precise description.

Linear relationship. x may increase as y increases in a linear manner so that x is *not* directly proportional to y . For example, if a straight line graph does not pass through the origin but cuts the ordinate at a distance a above it, then

$$x = a + Ky. \quad (2)$$

Then x/y is not constant and x is not directly proportional to y (Fig. 1b).

Similarly, if x decreases with increase of y in a linear manner x is *not* inversely proportional to y . In this case (Fig. 2)

$$x = a - Ky. \quad (3)$$

Inverse proportion. z may decrease as y increases in many ways but only when this happens so that zy is constant or

$$zy = P \quad (4)$$

is z said to be *inversely proportional to* or to *vary inversely as* y . The graph of z against y is then a hyperbola so arranged with respect to ordinate and abscissa that if $1/z$ is plotted against y a straight line which passes through the origin results; $1/z$ is then directly proportional to y (Fig. 1a). If, however, the graph of $1/z$

against y though linear does not pass through the origin but cuts the ordinate at a distance a above it, then

$$1/z = a + K_1 y, \quad (5)$$

and

$$zy = \frac{1 - za}{K_1}, \quad (6)$$

and zy is not constant. Then z is not inversely proportional to y although the curve of z against y is identical in shape (though not in position with respect to the fixed origin) to that expressed by $zy = P$ (equation (4)) and may have a similar biological significance (Fig. 1 *b*).

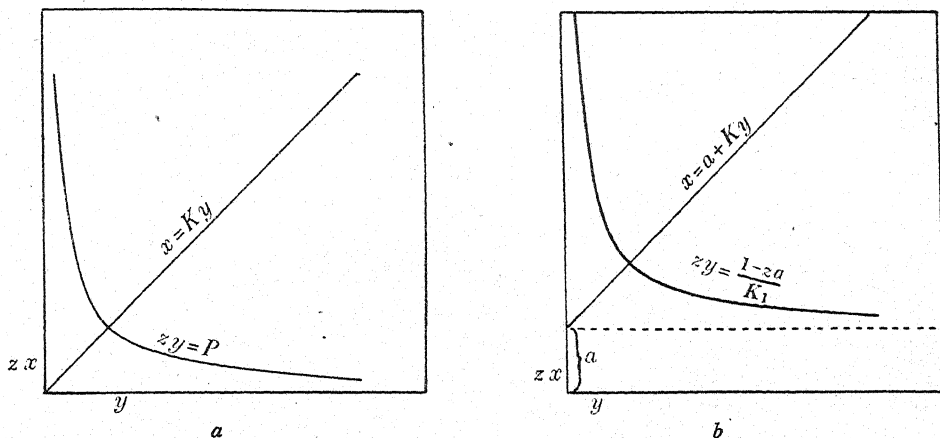


Fig. 1 *a, b*. Linear graphs and their hyperbola-shaped reciprocal curves.

(2) Longevity, saturation deficiency and water loss

In some insects the rate of water loss is linearly related to saturation deficiency over a wide range at constant temperatures, a separate graph existing for each temperature (see Ramsay, 1935 *a, b*; Johnson, 1940 *a*; and Fig. 3).

Let us denote the rate of water loss by R , saturation deficiency by S . Then the linear part of each graph could be expressed by the equation

$$R = a + KS.$$

R is, of course, not necessarily the rate of evaporation.

The slope of each graph (expressed by K) differs, and if the linear part of each graph is produced backwards so as to cut the ordinate it does so at a distance above the origin (expressed by a ; see Fig. 3). The higher the temperature, the greater the values of a and of K .

Thus, since all the graphs do not pass through the origin, R is not, in general, directly proportional to S even at constant temperatures. By definition, therefore, Dalton's law by itself would not hold good even at a constant temperature.

Consider, however, the simplest possible case; if

(i) R is directly proportional to S , and a is zero, then

$$R = KS \quad (\text{Figs. 1 } a, 3).$$

(ii) Insects all start with the same percentage water content and die after a certain critical quantity of water has been lost, i.e. water loss limits survival.

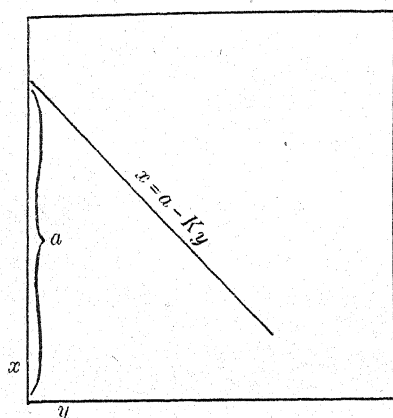


Fig. 2. Decrease of x with increase of y ; linear relationship.

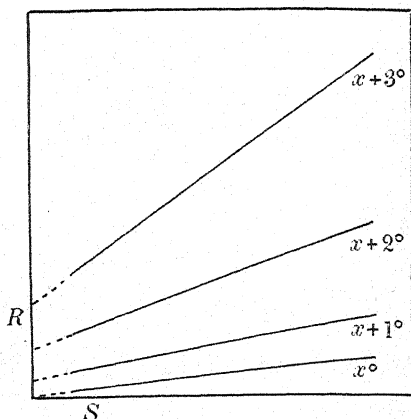


Fig. 3. R is directly proportional to S at constant temperatures only.

It follows that the greater the rate of water loss the shorter will be the length of life. Let us call length of life (longevity), L . L will, therefore, be inversely proportional to R (see columns below and Fig. 1a).

Sat. def. mm. S	Rate of water loss, mg./day R	Days for loss of 10 mg. water L
1	1	10.0
2	2	5.0
3	3	3.3
4	4	2.5
5	5	2.0
10	10	1.0

Thus

$$L = K_1 (1/R), \quad (7)$$

where K_1 is the quantity (or proportion) of water lost for death to occur,

and

$$R = KS,$$

then

$$L = K_1 / (KS),$$

and

$$LS = K_1 / K = \text{constant}.$$

It follows that

(iii) Longevity will be inversely proportional to saturation deficiency.

Thus when any two of the conditions are known to be as above, the third will also be as above.

In other terms, if it were true that Dalton's law of evaporation applied to insects and if water loss limits survival, then the length of life will be inversely proportional to or will vary inversely as the saturation deficiency. If, however, longevity varies inversely as the saturation deficiency and is limited by water loss, Dalton's law is *not* proved for the water may not all be lost by evaporation. And

when R is not directly proportional to S if the linear parts of the graphs of R on S expressed the evaporation rate the differences in position of the graphs at different temperatures (expressed by a) may possibly be due to a constant rate of water loss at all humidities at the same temperature and may have a cause other than evaporation (e.g. defaecation) which is affected by temperature though not by humidity.

Thus, if water loss limits survival and $R = a + KS$ as in Figs. 1b and 3, since

$$L = K_1 (1/R), \quad L = K_1/(a + KS).$$

But $LS = K_1 S/(a + KS)$ which is not constant on account of a . The graph of L on S is, however, identical in shape to the graph when a is zero and $LS = P$, and may have a similar significance as far as water loss is concerned.

In nature (Fig. 3) the 'positions' of hyperbolas of L on S over a limited range would of course depend primarily on a but also partly on K . K will also affect their shapes (Fig. 4). It would be very difficult without a large number of plotted points

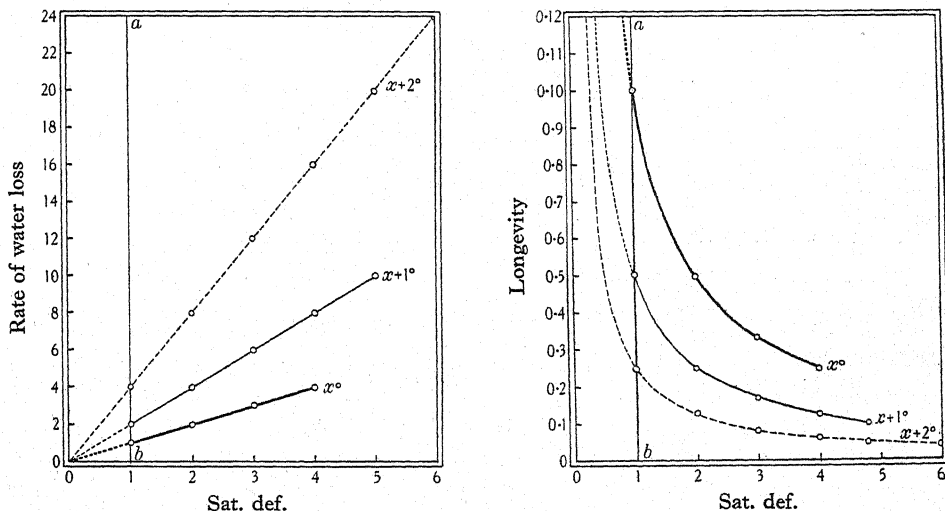


Fig. 4. Hypothetical curves of R and L against S at different temperatures with different values of K . Position and shape of hyperbolas change as K changes. From Johnson (1940a).

to ascertain if the longevity-saturation deficiency curves varied in position with respect to a fixed origin because of one or both of these factors. The relative positions of the curves is affected too by the state of nutrition of the insects (see p. 173).

I have postponed a consideration of those parts of the curves of R against S at very low saturation deficiencies—shown as dotted lines in Fig. 3—for it is uncertain how the graph is shaped at these high humidities with living insects. Do the curves pass through the origin after bending or do they bend but slightly or even continue in a straight line until they cut the ordinate?

In the rest of this paper the curves for longevity against saturation deficiency which have been given by various authors or taken from their data will be discussed in the light of the propositions which have now been stated.

III. THE WORK OF BACOT AND MARTIN ON THE LONGEVITY OF *XENOPSYLLA CHEOPIS*

The paper by Bacot & Martin (1924) on the rat flea, *Xenopsylla cheopis*, was the start for much of the recent work on water loss and survival in insects. Because of its historical importance and since misunderstandings have arisen from it we must reconsider the work and the conclusions.

The following statement and data (for 32° C.) are given by Bacot & Martin:

'By multiplying the saturation deficiency by the mean life a nearly constant number is obtained in the case of higher saturation deficiencies. This product, however, diminishes considerably at 4 mm. as saturation is approached. Indeed a departure must occur, for the fleas do not live for ever in saturated air when the saturation deficiency is zero.

'We conclude, however, that apart from this limitation, the mean life of the insect varies inversely as the saturation deficiency.'

R.H. %	Sat. def. mm.	Mean observed length of life in hours	Mean length of life \times Sat. def.
89	4	152	608
72	10	68	680
55	16	44	704
27	26	27	702

There are two points for criticism:

(1) L may be inversely proportional to S so that $LS = P$ and so that the straight line representing $1/L$ against S passes through the origin. But, as we have seen, there are many curves for L against S all of identical shape to the hyperbola where $LS = P$, for which LS is not constant (p. 153). These would be arranged one above the other so that the straight lines of $1/L$ against S would cut the ordinate at different distances a , a_1 , a_2 , etc., above the origin. All such curves might indicate biological happenings similar to those when $LS = P$, differing only in additional amounts of water or weight lost by means other than evaporation (p. 155).

Bacot & Martin's curve is actually of this type, but their value of a was so small that it passed unnoticed and LS appeared to be reasonably constant. They were incorrect, therefore, to state that L varied inversely as S .

Thus if two curves are fitted to Bacot & Martin's data, one by the method of least squares which takes account of a , and the other by means of Bacot & Martin's equation, $LS = P$, a much better fit is obtained when allowance is made for a . Moreover, there is then no departure from the expected curve at 4 mm. saturation deficiency as Bacot & Martin thought (Table 1).

(2) Bacot & Martin state that

'As the rate of drying also varies inversely as the saturation deficiency, this signifies that, at constant temperatures, the mean life of fleas apart from their host is inversely proportional to the rate at which they lose water by evaporation.'

Now it is obvious that the rate of drying of an insect would not vary inversely as the saturation deficiency. Unless there is a misprint in the above quotation it seems that Bacot & Martin could mean only that the *weight* of fleas decreased with time (see their Fig. 1 and table on the same page). If this is what happened they

Table 1. *Hyperbolas fitted to Bacot and Martin's data on longevity of fasting Xenopsylla cheopis at 32° C. Root mean square error, $(S \text{ obs.} - \text{exp.})^2/\text{no.}$ of observations, is smaller the closer the fit*

Sat. def. mm.	Mean longevity in days		
	Observed	Expected	
		<i>a</i> considered	<i>a</i> omitted
4	152	154.4	176
10	68	66.5	70.4
16	44	43.2	44.0
26	27	27.7	27.1
		1.49	12.06

Figures in black type denote root mean square error

confused a correlation of the rate of weight loss with survivorship at *one* saturation deficiency (10 mm., 32° C.) with the correlation of rate of water loss with longevity at different saturation deficiencies. They give no data for variation of rate of water loss at different saturation deficiencies and, therefore, apparently did not find the relationship between them. Neither did they find that water was lost only by evaporation as the above quotation implies.

IV. INVESTIGATIONS ON LONGEVITY AND MORTALITY IN RELATION TO SATURATION DEFICIENCY SINCE BACOT AND MARTIN

The problems of longevity and water loss from insects in relation to saturation deficiency of the atmosphere were reviewed by Buxton (1932) who stated (p. 288) that

'There is good reason for saying that the loss of water from an insect is proportional to saturation deficiency: but this is only the case within limits, which exist both at the dry and the wet end of the scale. Bacot & Martin (1924) showed that the duration of life of the flea was directly proportional to the dryness of the air....'

An incorrect use of the terms direct and indirect proportion exists here (cf. § II). Other workers have also used the terms incorrectly.

(1) *Leeson's work on fed and unfed rat fleas*

While Buxton's review was in the press Leeson (1932) published much data on *Xenopsylla cheopis* and other species of fleas which repeated and amplified Bacot & Martin's work. Leeson used Bacot & Martin's method for demonstrating the relationship between longevity and saturation deficiency (i.e. $LS=P$), and con-

cluded that there was no 'direct proportion' between them at any temperature. The term 'direct proportion' is again wrongly used here. Leeson's apparent contradiction of Bacot & Martin's conclusions was then, as Buxton (1932) pointed out, inexplicable. It can now be seen why this was so.

Table 2. *Longevity of Xenopsylla cheopis adults (sexes pooled), unfed and under 24 hr. old at start of experiment. Data from Leeson (1932, Table 1)*

The observed mean duration of life is shown together with the expected duration of life calculated by Leeson's method

$$L_{\text{exp.}} = \frac{\text{S.D. at 60\% R.H.} \times L \text{ at 60\% R.H.}}{\text{S.D.}},$$

and by the method of least squares for linear and hyperbolic relationships.

° C.	% R.H.	Sat. def. mm.	Mean observed longevity days	Leeson's method	Expected mean longevity Least squares	
					Linear	Hyperbola
37	0	46.9	1.3	—	1.33	—
	40	28.1	1.7	—	1.49	—
	60	18.8	1.3	—	1.57	—
	70	14.1	1.7	—	1.61	—
	75	11.7	1.3	—	1.63	—
	80	9.4	1.5	—	1.56	—
	90	4.7	1.9	—	1.69	—
					0.2076	—
30	0	31.7	3.5	—	3.82	—
	35	20.6	3.7	—	4.20	—
	50	15.9	4.2	—	4.37	—
	60	12.7	5.3	—	4.48	—
	75	7.9	4.4	—	4.64	—
	85	4.8	4.7	—	4.75	—
	95	1.6	4.4	—	4.86	—
					0.4351	—
23	0	21.0	3.7	2.5	3.64	—
	30	14.7	4.8	3.5	4.74	—
	40	12.6	4.9	4.1	5.11	—
	60	8.4	6.2	6.2	5.85	—
	75	5.3	6.5	9.8	6.39	—
	90	2.1	7.3	24.8	6.95	—
	95	1.1	6.5	47.3	7.13	—
					0.3175	—
18	0	15.5	4.8	—	4.78	5.83
	20	12.4	6.9	—	7.01	6.88
	75	3.9	13.3	—	13.12	11.11
	90	1.6	14.6	—	14.78	16.87
					0.1389	1.6592
12	0	10.5	14.8	6.84	13.13	15.23
	60	4.2	17.1	17.10	18.35	17.49
	75	2.7	19.1	26.20	19.59	18.70
	90	1.1	21.3	65.29	20.91	21.43
					1.0890	0.3574

Apart from the wrong use of terms, Leeson's method of calculating the 'expected' results was, like Bacot & Martin's, incorrect; for he also assumed that if a significant relationship existed *LS* would necessarily be constant.

Leeson took as the basis for his calculations the saturation deficiency at 60% R.H.,

and he argued that 'If survival was directly proportional to saturation deficiency' then

$$\text{Expected longevity at } X\% \text{ R.H.} = \frac{\text{sat. def. at } 60\% \text{ R.H.} \times \text{longevity at } 60\% \text{ R.H.}}{\text{sat. def. at } X\% \text{ R.H.}},$$

which is another way of saying that $LS=P$. This means, however, that L is inversely and not directly proportional to S . Leeson's expected and observed times were widely different, and he concluded that 'It is obvious that no relationship exists neither does it exist at any of the temperatures employed'.

If, however, straight lines are fitted to Leeson's data for *X. cheopis* by the method of least squares (Table 2) the expected and observed lengths of life are very close indeed. Thus the mean longevity of unfed fleas is linearly related to

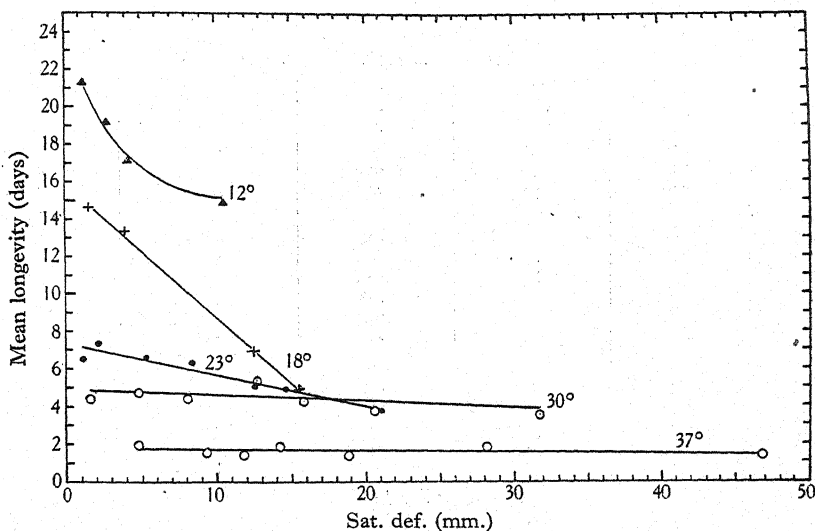


Fig. 5. Mean longevity of unfed *X. cheopis*. Data from Leeson (1932) with fitted lines (see Table 2).

saturation deficiency except at 12° C., where a hyperbola-shaped curve fits the data better than a straight line (Fig. 5). The same is true for *X. astia* and *Ceratomyxus fasciatus* except at 30° C., where a hyperbola gives a better fit (Table 3).

Thus even after the errors in curve fitting are eliminated Leeson's results still do not agree, in the main, with those of Bacot & Martin.

Now the above experiments were made with unfed fleas which were no more than 24 hr. old at the start of the experiment. Bacot & Martin, however, had used fleas of unknown age and varying states of nutrition. Believing that perhaps the nutrition factor had led to the disagreement, Leeson (1936) repeated his experiments using both fed and unfed fleas. As before, he failed to confirm Bacot & Martin's results; but this time his errors in curve fitting were largely responsible.

In Table 4 and Fig. 6 are the expected mean lengths of life when both straight lines and hyperbola-shaped curves are fitted by the method of least squares to Leeson's data. With unfed fleas and with those that had fed only once as adults

straight lines fit the data best, and this confirms the results found here for the 1932 data. The reverse, however, is true for fleas which fed repeatedly before starvation commenced. In this last case, therefore, Leeson's results do in fact agree with

Table 3. *Longevity of Xenopsylla astia and Ceratophyllus fasciatus adults (sexes pooled), unfed and under 24 hr. old at commencement.*
Data from Leeson (1932, Tables 5 and 7)

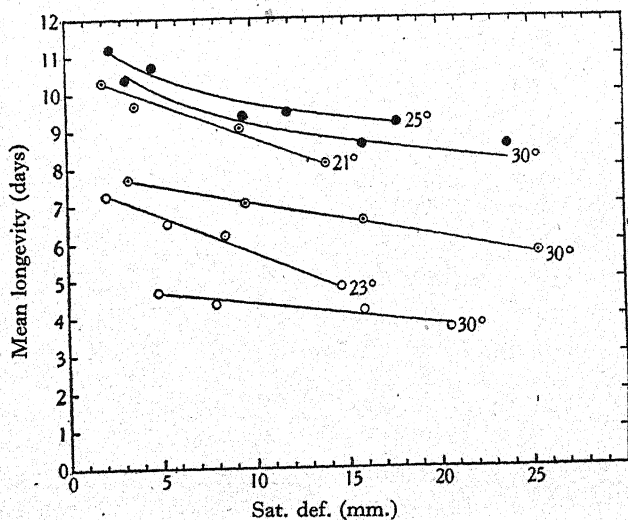
Other details as in Table 2

° C.	% R.H.	Sat. def. mm.	Mean observed longevity days	Expected mean longevity Least squares	
				Linear	Hyperbola
<i>Xenopsylla astia</i>					
30	0	31.7	4.8	4.20	4.21
	30	22.2	4.0	4.37	4.30
	50	15.9	4.4	4.47	4.38
	60	12.7	4.1	4.53	4.44
	75	7.9	6.0	4.61	4.57
	90	3.2	4.7	4.69	4.82
				0.6607	0.6600
23	0	21.0	4.0	4.62	6.72
	30	14.7	5.6	5.88	6.89
	60	8.4	7.2	7.15	7.16
	75	5.3	8.8	7.78	7.39
	90	2.1	7.9	8.42	7.87
	95	1.1	6.7	8.63	8.23
				0.9168	1.4940
18	0	15.5	6.1	6.45	6.81
	30	10.9	12.0	9.12	7.73
	75	3.9	11.5	13.18	11.22
	90	1.6	15.1	14.51	15.49
				1.7021	2.1777
<i>Ceratophyllus fasciatus</i>					
30	0	31.7	5.2	4.58	5.41
	30	22.2	6.3	6.99	6.20
	50	15.9	7.5	8.59	7.05
	75	7.9	8.7	10.62	9.22
	90	3.2	12.8	11.81	13.04
				1.1589	0.3358
23	0	21.0	6.5	6.47	6.38
	30	14.7	8.1	8.22	7.20
	60	8.4	10.1	9.98	8.70
	90	2.1	11.7	11.73	13.93
				0.0878	1.3924
18	0	15.5	8.0	8.00	10.65
	30	10.9	11.9	11.48	11.52
	75	3.9	16.3	16.76	14.52
	90	1.6	18.8	18.50	17.73
				0.3457	1.6941

those of Bacot & Martin; for although, as in Bacot & Martin's work, longevity is not inversely proportional to saturation deficiency at a constant temperature, the curves are hyperbola-shaped. The possible reasons why some curves are linear and others curved are discussed below (p. 174).

Table 4. *The longevity of Xenopsylla cheopis. Linear and hyperbolic curves fitted to Leeson's (1936) data. Sexes pooled. See Fig. 6*

° C.	% R.H.	Sat. def. mm.	No. of fleas	Mean longevity (observed)	Expected longevity	
					Linear	Hyperbola
Unfed fleas						
23	30	14.7	154	4.8	4.83	—
	60	8.4	113	6.2	6.05	—
	75	5.3	98	6.5	6.66	—
	90	2.1	140	7.3	7.28	—
30	35	20.6	60	3.7	3.78	—
	50	15.9	107	4.2	4.06	—
	75	7.9	68	4.4	4.53	—
	85	4.8	75	4.7	4.71	—
Fleas once fed						
21	25	13.9	67	8.1	8.15	8.35
	50	9.3	58	9.1	8.96	8.72
	80	3.7	43	9.7	9.94	9.63
	90	1.9	130	10.3	10.26 0.14	10.35 0.23
30	20	25.4	97	5.7	5.71	—
	50	15.9	65	6.6	6.56	—
	70	9.5	64	7.1	7.13	—
	90	3.2	102	7.7	7.70 0.03	— 0.25
Fleas fed repeatedly						
25	25	17.8	90	9.2	8.99	9.19
	50	11.9	93	9.5	9.81	9.57
	80	4.7	56	10.7	10.82	10.49
	90	2.4	298	11.2	11.14 0.20	11.22 0.11
30	25	23.8	39	8.6	8.16	8.23
	50	15.9	64	8.6	8.98	8.66
	70	9.5	30	9.4	9.63	9.23
	90	3.2	120	10.4	10.28 0.32	10.56 0.22

Fig. 6. Mean longevity of *X. cheopis*. Data from Leeson (1936) with fitted lines (see Table 4).
● fed repeatedly; ◐ fed once; ○ unfed.

(2) *Kirkpatrick's (1923) data on the longevity of the bug, Oxycareus hyalinipennis*

In trying to decide if Bacot & Martin's results applied to other insects, Buxton (1932) analysed Kirkpatrick's large quantity of data on the survival of *Oxycareus hyalinipennis* adults under different conditions of temperature and humidity. Buxton (1932, p. 286), writing of Dalton's law, supposed that 'according to this law the loss of water evaporated is proportional to the saturation deficiency. If we take the

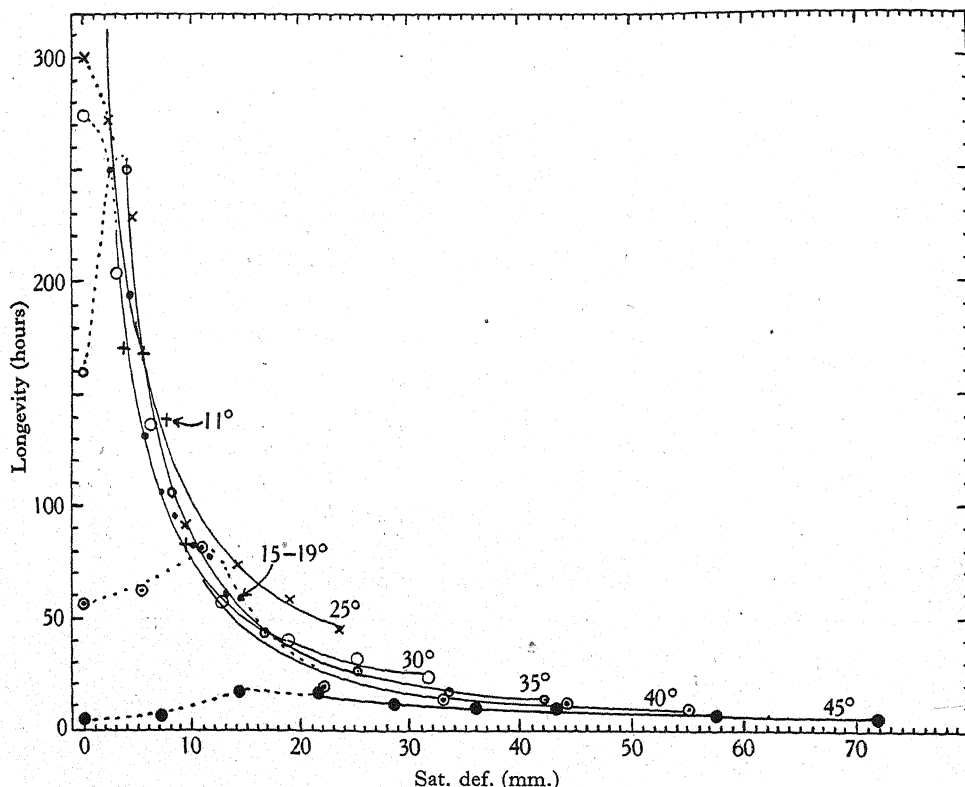


Fig. 7. Longevity of *Oxycareus hyalinipennis*. Data from Kirkpatrick (1923) with fitted hyperbolas (see Table 5). Dotted lines run through points not included in curve fitting.

mean duration of life at a particular saturation deficiency and assume the validity of Dalton's law, we can calculate what the duration should be at any other saturation deficiency and the same temperature.' The expected values for longevity were calculated for *O. hyalinipennis*; the time for 50% mortality was taken from Kirkpatrick's graphs (no numerical data having been published from which means could have been calculated), and the method of calculation was identical to that used by Leeson for rat fleas. Buxton's conclusions were that at 17, 25 and 30° C. there was a fair agreement between the observed and expected figures, and this also applied at 35, 40 and 45° C. below 80% R.H. At 11° C., however, there was no such agreement.

Now if the time for 50% mortality is plotted against saturation deficiency we see (Fig. 7) that except for some high humidities the curves are all apparently shaped like hyperbolas and lie so close to each other that they can be represented by almost a common curve. If hyperbolas are fitted by the method of least squares to those parts of each curve below certain high humidities there is a very much closer fit than Buxton supposed (Table 5). This suggests two possibilities.

(a) Although the constant a cannot be neglected and therefore it is unlikely that R will be directly proportional to S , R may be linearly related to S at each temperature, except at very high humidities, provided that water loss is the limiting factor in survival (a factor on which there are no data).

(b) The closeness of the curves to each other suggests that the temperature has little direct effect on longevity and is possibly without effect on the rate of water loss. Kirkpatrick's bugs were, however, caught wild before the experiment, and differences in their state of nutrition which could influence the positions of the graphs might have existed (see p. 173).

(3) *MacLeod's (1935) data on the longevity and water loss of the tick,*
Ixodes ricinus

MacLeod found that the product of survival time and saturation deficiency was not constant, but that its value increased with decrease in humidity. He took this as an indication that 'with decreasing humidity death becomes due more and more to loss of water, the heat-lethal effect having increasingly less time to operate, and, consequently, being responsible for an ever lessening share in producing death'.

Now although the mean length of life of *Ixodes ricinus* is not inversely proportional to saturation deficiency it may be related to it in such a way that the reciprocal of L (and, therefore, the rate of water loss too) is linearly related to S . Thus, (1) if with the experimental results a hyperbola can be fitted to longevity against saturation deficiency and (2), if also a linear graph expresses the relationship of R to S then it is reasonable to suppose that (3) MacLeod's data is consistent with the hypothesis that longevity is limited ultimately by water loss. Both (1) and (2) above, can be shown to obtain with MacLeod's data.

(a) *Longevity (Table 6, Fig. 8a).*

It is obvious that longevity is related to saturation deficiency in a fairly linear manner. If, however, the point at the lowest saturation deficiency is omitted (and this seems legitimate since other factors than water loss may limit longevity at very high relative humidities, see p. 174) the remaining points fall on a hyperbola.

(b) *Rate of water loss (Table 7, Fig. 8b).*

MacLeod, like Mellanby (1932), rightly concluded that if R/S is not constant R is not directly proportional to S and, therefore, Dalton's law alone does not hold good. But, we have seen that if in addition to water loss according to Dalton's law there was also a loss due to defaecation which, though independent of humidity, might vary with temperature, then R against S would be linear and with much the

Table 5. *Data for 50% mortality in Oxycarenus hyalinipennis. Kirkpatr (1923) data*

° C.	Sat. def. mm.	% R.H.	Observed time hr.	Expected time (hr.)	
				Buxton's estimate	Fitted hyperbola
11	9.8	1	82	68	99.6
	7.8	20	138	85	117.4
	5.9	40	168	113	143.4
	3.9	60	170	170	192.9
				38.9	21.6
17 (15-19)	14.5	1	58	53	58.3
	13.0	10	60	58	64.5
	11.6	20	78	66	71.7
	10.1	30	82	75	81.5
	8.7	40	96	88	93.6
	7.2	50	106	106	111.5
	5.8	60	132	132	136.2
	4.3	70	194	198	179.6
	2.9	80	250	264	258.7
				7.4	6.6
25	23.7	1	45	37	47.3
	19.0	20	58	46	56.8
	14.2	40	74	62	72.2
	9.5	60	92	92	100.7
	4.7	80	228	184	179.9
	2.4	90	272	376	313.3
*	0	100	300	—	—
				46.7	26.2
30	31.7	1	23	22.4	24.5
	25.4	20	32	28	30.3
	19.0	40	40	37	40.1
	12.7	60	56	56	59.1
	6.3	80	136	112	116.3
	3.2	90	204	224	223.5
*	0	100	274	—	—
				12.8	11.6
35	42.0	1	14	17.6	13.2
	33.4	20	16	22	17.8
	25.2	40	26	27	25.5
	16.8	60	44	44	42.9
	8.4	80	106	88	104.3
	4.2	90	250 ?	176	253.6
*	0	100	158	—	—
				3.1	1.9
40	55.1	1	8	7.2	7.1
	44.1	20	11	9	9.7
	33.0	40	13	12	14.5
	22.0	60	18	18	25.4
	11.0	80	82	36	66.3
	5.5	90	62	—	—
*	0	100	56	—	—
				20.6	7.8
45	71.9	1	5.0	4.12	5.1
	57.5	20	6.5	5.15	6.2
	43.1	40	8.5	6.00	8.1
	35.9	50	9.1	8.20	9.5
	28.7	60	10.3	10.30	11.6
	21.5	70	16.4	13.70	15.1
*	14.3	80	17.4	—	—
	7.1	90	6.3	—	—
*	0	100	5.0	—	—
				1.5	0.8

* = omitted from curve fitting (hyperbola).

same biological significance as if Dalton's law alone operated. R/S in this case would not be constant.

Now the daily weight-loss/saturation deficiency with nymphs and non-tracheate larvae of *I. ricinus* was not constant but was highest in relatively wet air. MacLeod, therefore, thought that relatively more water was lost in wet than in dry air in unit time, but that at 50% R.H. and less (except in very dry air) R was 'roughly proportional' to S .

The situation is simplified by plotting R against S with data for nymphs (those for larvae are insufficient). Then if the mean of the rates of water loss over several days is taken and not the daily loss it is clear that the relationship of R to S is

Table 6. *The observed longevity of unfed nymphs of Ixodes ricinus at 24° C. and the fitted values for linear and hyperbolic curves. From data of MacLeod (1935). See Fig. 8a*

% R.H.	Sat. def. mm.	Longevity in hours		
		Observed	Expected	
			Linear	Hyperbola
70	12	32	—	—
50	20	27	26.3	27.2
30	28	20	21.5	20.2
10	36	18	16.8	16.3
0	40	14	14.4	15.0
$S(\text{obs.} - \text{exp.})^2$				4.5
				3.8

Table 7. *Mean daily weight loss (percentage original weight) of unfed nymphs of Ixodes ricinus at 25° C. From data of MacLeod (1935). See Fig. 8b*

% R.H.	Sat. def. mm.	No. of days	Mean daily weight loss
70	7.1	6	5.49
50	11.9	5	6.03
30	16.6	4	9.74
10	21.3	3	12.28

probably linear over most of the humidity range used. R is not directly proportional to S , but it is linearly related to S which is just as significant as a direct proportion.

Survival is thus perhaps limited by water loss over a wider humidity range than MacLeod thought; high humidities do apparently have an adverse effect on vitality although MacLeod supposed that they did not; and there is no need to invoke a 'heat-lethal effect'.

(4) *Longevity and the rate of water loss with the bed-bug, Cimex lectularius*

The longevity of *Cimex* in relation to rate of water loss has been fully discussed elsewhere (Johnson, 1940a). A short summary is necessary here, for slight corrections have to be made to previous statements.

The information available for *Cimex* is similar to that of MacLeod for *Ixodes*, but there is more of it. The arguments and conclusions are also similar and they illustrate the theoretical principles discussed in § III. At all temperatures between

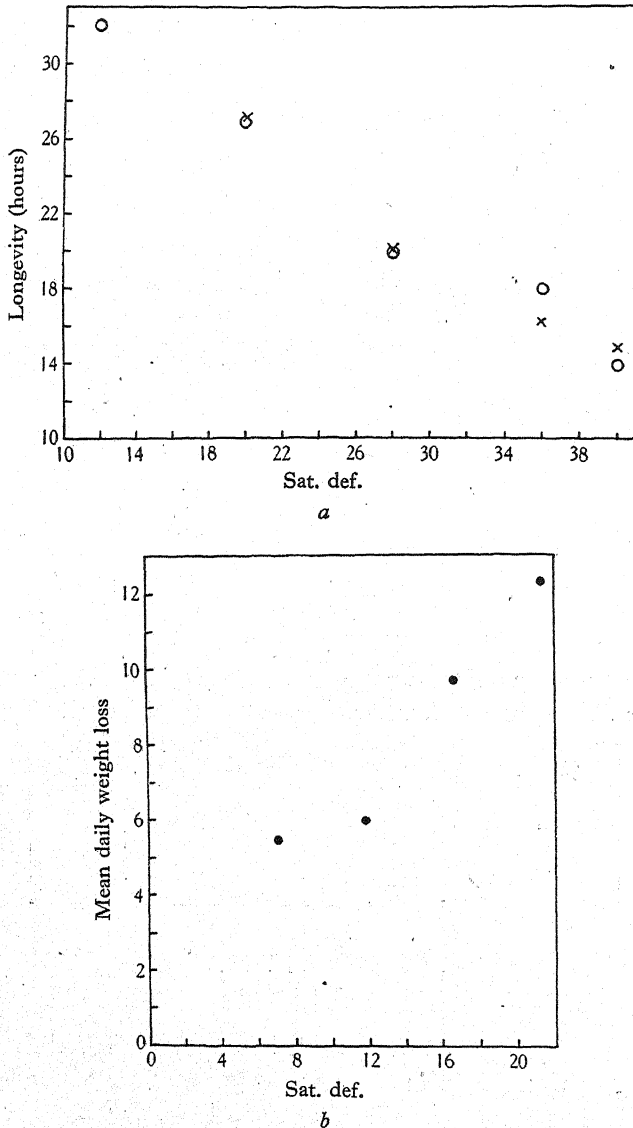


Fig. 8. *a*, Longevity of unfed nymphs of *Ixodes ricinus* at 24° C. O observed; x hyperbola fitted to points at four highest saturation deficiencies (see Table 6). *b*, Mean daily weight loss of unfed nymphs of *I. ricinus* at 25° C. (see Table 7).

7 and 36° C. both longevity and the reciprocal of the rate of water loss in relation to saturation deficiency have been shown to be well represented by hyperbola-shaped curves. Mellanby's data on the rate of water loss (1932), when treated as

MacLeod's, show that within reasonable limits of experimental error the rate of water loss, though not directly proportional to saturation deficiency is linearly related to it at constant temperatures except at very high relative humidities (Johnson, 1940a).

The corrections to my 1940 paper are as follows:

I stated that though R/S is not constant R may still be directly proportional to S , and that Dalton's law may still apply at a constant temperature if all the water was lost by evaporation above certain high humidities. This, of course, is wrong, for R is directly proportional to S only when the straight line passes through the origin and R/S is constant. But it is linearity rather than a direct proportion which is significant to the argument set out in this and in the former paper, and the graph may still be linear even if R/S is not constant.

I also stated that the departure of the graph of R against S from linearity at very low saturation deficiencies may be due to defaecation (in addition to evaporation) operating more at high than at low humidities. This view now requires some modification (see p. 155); for the graph can be better interpreted by supposing that a constant rate of water loss by defaecation exists which is unaffected by humidity.

(5) *Martini and Teubner's data on the longevity of Theobaldia annulata*

These authors published their data (1933) on the mean length of life of *Theobaldia annulata* at different temperatures and humidities. The hyperbolic shape of the curves of longevity against saturation deficiency suggests that the rate of water loss may be linearly related to saturation deficiency at constant temperatures. These authors' data on other mosquitoes indicate similar longevity-saturation deficiency curves which are less symmetrical and apparently more influenced by temperature than those of *Th. annulata*.

(6) *Grossman's (1930) data on the longevity of the cotton boll weevil, Anthonomus grandis*

Buxton (1932) considered that these data supported the view that saturation deficiency is the effective measure of water loss. There are two technical points that make Grossman's data of uncertain value:

(1) The weevils, captured in the field, were of unknown age at the start of the experiments.

(2) The desiccators containing them were aerated twice daily, and it may take several hours for the air remaining inside to regain its former humidity unless a humidified air stream is passed through.

Grossman worked at 21° and 27° C.; records at 27° C. were, however, made at rather wide and irregular intervals of time, and are therefore less useful than those at 21° C., where the time intervals were much shorter. I have found the mean times of survival from the data. For unfed weevils at 21° C. a hyperbola-shaped curve fits the data well if the observations at 98 and 100% R.H. are omitted. With fed insects the data are too scanty to draw definite conclusions, but the curve seems to

plotted with these last two variables as co-ordinates. Across the diagram were drawn lines of equal saturation deficiency, and it was found that some of these very nearly coincided with the lines of equal mortality. It was concluded, therefore, that mortality was determined by saturation deficiency (Buxton, 1931, p. 29). Other workers (Leeson, 1932; Maercks, 1933; Mellanby, 1935) used this graphical method; but its implications have not been properly considered. There is, for example, the strange fact that egg or pupal mortality seems to bear a similar relation to saturation deficiency whether the time for mortality enters into the calculation

Percentage of *Melanoplus atlanis* eggs hatching at different constant temperatures and constant relative humidities

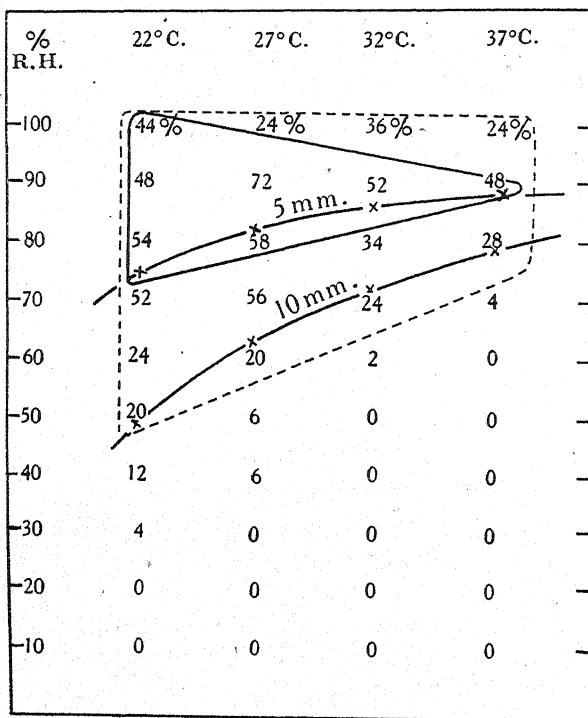


Fig. 10. Mortality of *Melanoplus* eggs in relation to saturation deficiency. From Buxton (1932) and Parker (1930).

(Mellanby, 1933) or whether it is neglected (Buxton, 1931, 1932). It is well, therefore, to examine the method closely.

In a diagram whose co-ordinates are temperature and relative humidity, lines can be drawn through points of equal saturation deficiency (Fig. 10). Consider any one such line; it will represent the same saturation deficiency at *different temperatures*. If a line joining equal mortalities coincides with it, it will mean that at *one* saturation deficiency *but at different temperatures* mortality is the same; i.e. $\text{sat. def.} \times \text{mortality} = \text{constant}$, *irrespective of temperature*. It is important to realize, however, that the relationship expressed by this equation is not of a general nature and applies only to a *single* saturation deficiency. It does not express the relationship

of mortality to different saturation deficiencies. For whatever the relationship between mortality and different saturation deficiencies the line joining equal mortalities would always coincide with a line of equal saturation deficiency provided temperature was without appreciable effect on mortality.

In order to ascertain the effect of different saturation deficiencies on mortality, and thereby to speculate on Dalton's law, we must plot percentage mortality against different saturation deficiencies. Let us examine Parker's data on *Melanoplus* in this way.

It will be seen from Fig. 11 that there are optimum humidity conditions for survival but in air drier than optimal the graphs for mortality lie very close together for all the temperatures used. It is, therefore, obvious that humidity is more

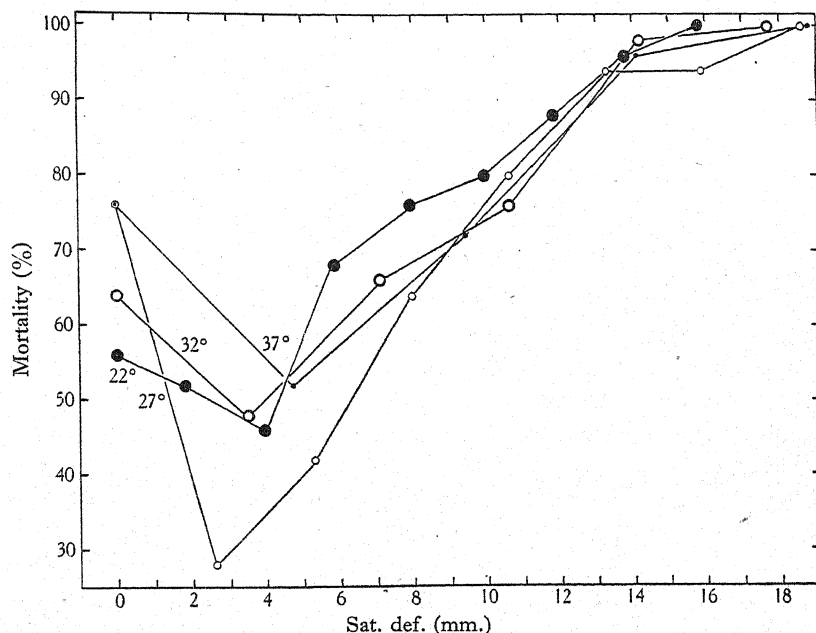


Fig. 11. Mortality of *Melanoplus* eggs plotted against saturation deficiency. Data from Parker (1930).

important than temperature for mortality and that the four graphs above a saturation deficiency of 4 mm. could be considered almost as one. Thus each saturation deficiency will have roughly only one corresponding mortality whatever the temperature, and the line joining equal mortalities will coincide with a line joining the corresponding saturation deficiency at different temperatures when temperature and relative humidity are the ordinates.

Thus Parker's data shows that the mortality of *Melanoplus* eggs is related to saturation deficiency more simply than to relative humidity because temperature has so small an effect on mortality. The relation of mortality to saturation deficiency is, however, in itself not a simple one.

What interests us now is the physiological significance of these facts and how they throw light on water loss from the eggs. When Buxton (1931, p. 30) stated

that 'the law of saturation deficiency applies, within certain temperature limits both to eggs and to adult insects of several different orders', he meant that the rate of water loss from these organisms was directly proportional to saturation deficiency within the limits of temperature mentioned. But as far as the data on *Melanoplus* eggs go this cannot be said to have been demonstrated. For, proceeding on the assumption that death occurs when a critical quantity of water is lost, what we require to know is *how long* the eggs survived rather than how *many* survived.

Mellanby (1933) was the first worker to realize the importance of time in experiments such as these. Working with the water loss from larvae of the flea, *Xenopsylla cheopis*, and the success with which they pupated, Mellanby stated that 'if a flea larva loses water at a rate proportional to the saturation deficiency of the air—and it can only lose a certain amount of water and survive—it will be able to withstand a greater saturation deficiency at a higher temperature than a low, because it will be losing water for a shorter time' (i.e. the higher the temperature the shorter the larval period, over which water loss is possible). Thus Mellanby concluded that a constant should be obtained if duration of larval stage was multiplied by the highest saturation deficiency (lowest humidity) which allows pupation at each temperature. He found, moreover, that this constant did occur when the hypothesis was tested experimentally.

Now with the larva of *Xenopsylla* we have a well-defined case; for since, as Mellanby showed, the pupa is resistant to water loss, all loss of water which occurs between hatching from the egg and successful pupation occurs from the larva and the prepupa. Since this water loss proceeds, presumably, during the entire length of the larval and prepupal periods, the period during which water is lost is the same as that between hatching and pupation—period which varied with temperature and which was easily found.

With insect eggs it is also essential to take into account the period during which water is lost as well as the saturation deficiency if the true measure of evaporating power of the environment is to be considered. But it seems unsafe to assume that the time during which water is being lost is, at any temperature, the total duration of the egg stage at that temperature, although it may be a fairly constant proportion of that time, and therefore some degree of constancy would be expected if that time was used. If, however, the period of water loss is short (e.g. before the 'middle egg membrane' or before the cuticle is laid down) in comparison with the total egg period, then considerable deviations from a constant may be expected if the total incubation period is used as the time factor. This is possible because the period of water loss may not bear a constant relation to the total incubation period at different temperatures; it has, in fact, been pointed out (Johnson, 1940*b*) that the thermal constants for early embryonic processes may be different from the later ones.

The apparently strange fact that Dalton's law was supported by Buxton's results, which were obtained without taking time into account and also by those of Mellanby which considered time, is now clear. For actually Buxton's diagram did not show how mortality was related to saturation deficiency, but only how it was related to the same saturation deficiency at different temperatures.

It is well to consider at this stage the data of Maercks (1933) for the eggs of *Habrobracon juglandis*. This author kept *Habrobracon* eggs at many combinations of temperature and humidity, and when he plotted mortality against saturation deficiency found that there was no simple relation between the two variables (Fig. 12). At each temperature there was an optimum humidity of approximately 80% R.H., and it was mainly because of this constant value that Maercks concluded that mortality was related more closely to relative humidity than to saturation deficiency.

Gunn (1935) and Mellanby (1935) criticized this work each on much the same grounds, though independently. Both these workers maintain that the time during which water is lost should be taken into account if Dalton's law is to be properly tested. Gunn plotted mortality against the product of saturation deficiency and developmental time and concluded that over a certain prescribed temperature range Dalton's law did not appear to be invalid. Mellanby came to much the same conclusion and stated that the mortality is much more closely related to saturation deficiency than to relative humidity for *Habrobracon* eggs when the time factor is introduced.

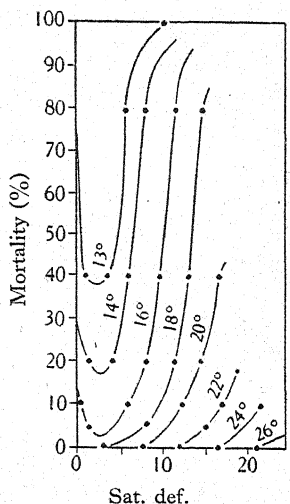


Fig. 12. Mortality of *Habrobracon* eggs plotted against saturation deficiency. From Maercks (1933).

As pointed out above, however, the period of water loss may not be the same as the total incubation period; and if Gunn and Mellanby could have used this period of water loss instead of the developmental time their results might have been even more consistent with Dalton's law. Unfortunately, this period of water loss is not known, and it would be difficult to ascertain it experimentally. It may, however, be possible to take arbitrary periods of time for each temperature and multiply these by the appropriate saturation deficiency until a constant was obtained. Eggs could then be exposed to different combinations of temperature and humidity for different periods and then incubated at constant conditions. Minimum periods of exposure which produce mortality could be found, and if these were similar to the periods which gave a constant value when multiplied by the appropriate saturation deficiency there would be good grounds for supposing that this period was that over which water was lost.

V. THE POSITIONS OF THE LONGEVITY-SATURATION DEFICIENCY CURVES DUE TO FEEDING

Fed insects live longer than unfed insects, other conditions being equal. The curve of longevity against saturation deficiency for fed insects will, therefore, lie above the curve for unfed insects. The curves for fed and unfed fleas (Leeson, 1936) differ in shape as well as in position, but with *Cimex* (Johnson, 1940a) the shape is

not greatly altered and a hyperbola fits both curves well. With *Cimex* and others like it, therefore, there are at least two possibilities:

(1) That feeding affects the slope of the curve of R on S and perhaps also the values along the co-ordinates at which the curve becomes linear.

(2) R is unaffected by feeding.

It seems *a priori* that the second case would be more likely, and a much more probable explanation of the difference in position of the longevity-saturation deficiency curves for fed and unfed insects is that the presence of food in the gut merely increases the amount of water available to the insect, that water loss remains the limiting factor in survival, and that $LR = K$ at different saturation deficiencies if all the insects are in the same nutritional state at the start of the experiment. K will, of course, differ for longevity-saturation deficiency curves which are different in position along the ordinate and represent different nutritional states.

The differences in position of the curves which are due to variation in slope of the curve of R on S have been discussed on p. 155.

VI. DEVIATIONS OF THE LONGEVITY-SATURATION DEFICIENCY CURVES FROM THE HYPERBOLA

It is obvious that a true hyperbolic relationship between longevity and saturation deficiency would not exist in nature. For, although along the abscissa the arm of the hyperbola is limited only by the greatest possible value of saturation deficiency at that temperature, the arm along the ordinate would not be asymptotic since insects are not immortal. Thus deviations from the hyperbola must occur at high relative humidities (low saturation deficiencies). This may be due to or correlated with

(1) High humidity itself being unfavourable to survival; e.g. when accumulation of metabolic water due to a low rate of water loss may lead to a dropsical condition (Buxton, 1932).

(2) Non-linearity of the graph of R on S at low saturation deficiencies.

(3) Food in the gut becoming exhausted before the water (particularly in unfed insects).

Other deviations may be as follows:

(4) Where both hyperbola and straight lines represent the relationship of L to S equally well; e.g. when only the tail of the hyperbola exists.

(5) Where straight lines alone fit the longevity-saturation deficiency data and hyperbolas do not.

(6) Where even at intermediate humidities the rate of water loss is not linearly related to saturation deficiency, although water loss is the limiting factor in survival.

When (4) above obtains, the straight line may be merely the tail of the hyperbola as with *Cimex* (Johnson, 1940*a*) and perhaps with Leeson's fleas. When (5) obtains, and a straight line represents the relationship of L to S over the whole range of humidity, a hyperbola may give a good fit for that part of the curve below very high humidities (e.g. Leeson, 1936; MacLeod, 1935). This may be due to either (1), (2) or (3) above. In Leeson's work the longevity-saturation deficiency curve was

hyperbolic for fed and linear for unfed fleas. It may be that here either (1) or (3) above is responsible. Unfortunately, Leeson did not find the relationship of R to S , and we cannot find if $LR = K$ which would obtain if water loss was the limiting factor in survival (see p. 169). With *P. japonica* (Ludwig, 1937) this method can be applied; for the insects must all start with the same water content, and the experiments for R and L must be made preferably with the same individual insects.

Finally, it may be said that, although water loss may appear to be the limiting factor in survival, water loss may not be the actual cause of death but only completely correlated with that cause.

VII. CURVE FITTING AND THE COMPARISON OF DATA

It will have been seen that by plotting longevity against saturation deficiency certain types of curve are obtained, e.g. the hyperbola. The fact that similar curves would be obtained if (1) the rate of water loss was directly proportional to saturation deficiency, and (2) if water loss limited survival, does not of course mean that a hyperbolic curve of longevity against saturation deficiency demonstrates the truth of Dalton's law. In fact it may lead to the belief that the rate of water loss is *not* directly proportional to saturation deficiency even at a constant temperature if the longevity-saturation deficiency curves are different in position along the ordinate at different temperatures. The hyperbola does, however, *suggest* that the rate of evaporation might change at a constant rate with change in humidity over a considerable humidity range at a constant temperature.

But apart from the causes and the significance of a hyperbolic relationship between longevity and saturation deficiency it is valuable to be able to establish such a generalization and to be able to compare the work of different authors, who use different insects or employ different techniques.

I have discussed only some of the more relevant papers; many I have not mentioned owing to the similarity or to the inadequacy of their data. Many data show that a hyperbola-shaped curve represents the relationship of longevity to saturation deficiency (e.g. Gosswald, 1938, with ants); others such as those of Payne (1929) and Maldwyn Davies (1928) obviously show neither linear nor hyperbolic curves over a considerable humidity range. These latter results may be due either to special factors in the insects themselves or to faulty technique.

It must be emphasized that many of the results discussed in this paper have been obtained with very few data, and points on graphs have been scanty. It is therefore very probable that some of the curves considered in this paper could each be expressed by more than one type of formula, and those that appear to be hyperbolic would best be fitted by another type of curve had more points been gathered by the investigator. This is especially true for longevity at very low saturation deficiencies, where the curve is steep and quite crowded points are necessary for reasonable accuracy.

VIII. SUMMARY

1. If the relationship between rate of water loss from an insect and saturation deficiency is linear and survival is limited only by water loss, then the curve of longevity against saturation deficiency is hyperbola-shaped. The position of this curve with respect to the co-ordinates is, however, affected by other factors, which are discussed.

2. The work of Bacot & Martin (1924) on the longevity of the rat flea, *Xenopsylla cheopis*, is reviewed in the light of the above hypothesis: errors in curve fitting are revealed which make it necessary to modify some of Bacot & Martin's conclusions.

3. Selected data from various authors subsequent to Bacot & Martin are considered. The discrepancies, real and apparent, between Leeson's results with rat fleas and those of Bacot & Martin are analysed, and it is pointed out that these were to some extent due to errors in curve fitting. The hypothetical relationship between longevity, rate of water loss and saturation deficiency set out in the first part of this article are illustrated by Ludwig's (1937) data for *Popillia japonica*; the linear relationship between $1/\text{longevity}$ and the rate of water loss demonstrates the hypothesis that water loss limits survival with this insect. Other less-complete data are also discussed.

4. Various types of longevity-saturation deficiency curves are described and the causes which probably account for such variations.

5. The relationship of pupal or egg mortality to saturation deficiency cannot by itself throw light on the variation of rate of water loss with saturation deficiency; the time factor must also be taken into account. Lines of equal mortality may coincide with lines of equal saturation deficiency (the co-ordinates being relative humidity and temperature), so that the product of mortality and saturation deficiency is constant at any one temperature; but this indicates only that temperature is without effect on mortality and does not throw light on the relation of mortality to different saturation deficiencies. Such coincidence of lines cannot, therefore, be taken as proof that Dalton's law operates.

This work was done while I held the Avebury Studentship in the Department of Entomology, London School of Hygiene and Tropical Medicine, and I am greatly indebted to Prof. P. A. Buxton for his great kindness and ready help. I also thank Drs W. J. Martin and D. L. Gunn for their advice and criticism.

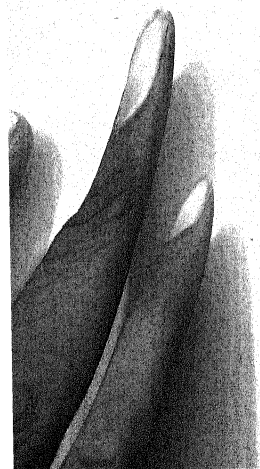
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ERRATUM

A correction should be made in Fig. 1*b* of the article by C. G. Johnson on p. 153 of the present volume, the position of the hyperbola being incorrect: it should intersect the ordinate when $z = \frac{1}{a}$ and should be asymptotic to the abscissa.



THE EVOLUTION AND ANATOMY OF THE CEREBELLUM

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I. INTRODUCTION

IN recent years descriptions of the form and development of the cerebellum in Amphibia, reptiles and certain primitive mammals have led to a 'somewhat modified morphologic conception of the cerebellum' (Larsell, 1937). Experimental analysis, both anatomical and physiological, in so far as it has proceeded, has demonstrated the soundness of these ideas of cerebellar morphology. It seems desirable therefore to bring together important recent contributions to our knowledge of the anatomy of the cerebellum. For the purpose of a terminological background, the contributions of investigators whose work has had an influence on present-day cerebellar terminology will be summarized briefly. The morphology of the cerebellum in submammalian forms will be presented, followed by the recent descriptions of the development of the mammalian cerebellum. Finally, important contributions to our knowledge of cerebellar fibre connexions in mammals will be summarized.

II. CEREBELLAR TERMINOLOGY

An early understanding of cerebellar morphology was hampered by efforts of anatomists to fit the folial pattern of the organ in all mammals into that morphological monstrosity, the human cerebellum. As a result, terms from human anatomical nomenclature have been applied to the lobes and fissures in all mammals. In addition to these terms borrowed from human anatomy (Fig. 1), various authors,

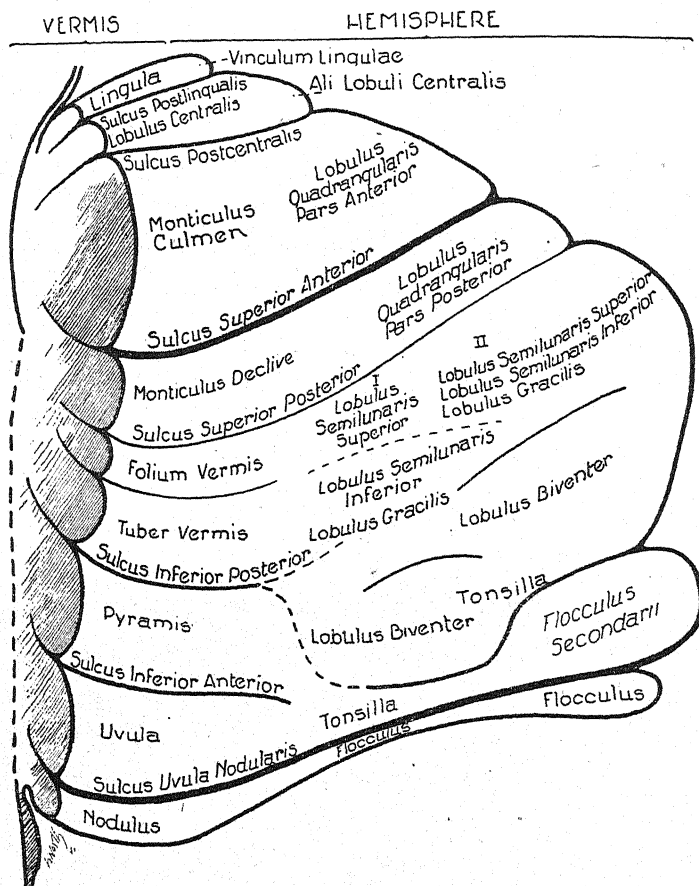


Fig. 1. Schema of the mammalian cerebellum with the lobes and fissures named according to B.N.A. terminology. In this and subsequent diagrams the cerebellum is represented as sectioned in the mid-sagittal plane and folded out so that all folium are seen on the surface. The homologies between the human and lower forms are not yet clear for the posterior parts of the lateral lobes. The terms under column I are as given by Bolk and followers, and under II as given by Ingvar and followers.

sensing the difficulties of applying such a terminology generally, endeavoured to simplify the situation by devising a new nomenclature independent of the older terms of the human anatomists. None has received universal acceptance, but each has contributed something to present cerebellar terminology.

One of the first and best comparative and developmental studies of the mammalian cerebellum was that of Stroud (1895). In it may be found a rather complete

review of previous work, most of which had been concerned with the description of relatively late developmental stages in the human. Stroud clearly pointed out that the cerebellum has a bilateral origin from the alar plates of the rhombencephalon. He recognized that the flocculus and paraflocculus were separate, but failed to appreciate the absence of morphological unity in the vermis which he made a fundamental division of the cerebellum.

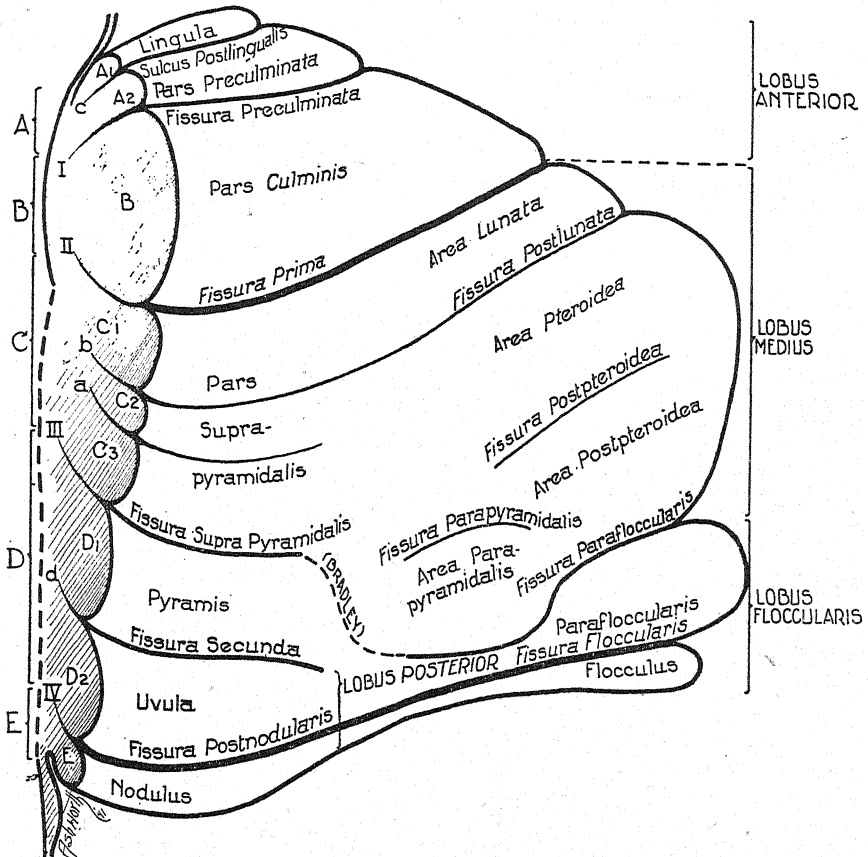


Fig. 2. Schema of the mammalian cerebellum with the lobes named according to the terminology of Elliot Smith (1902-3). The figures and letters shown on the mid-sagittal cut surface are those applied to the cerebellar divisions by Bradley (1903-4).

The investigations of Elliot Smith (1902, 1903 *a*, 1903 *b*), however, became more widely known and had great influence on subsequent work (Fig. 2). He studied the adult brains of many mammals and developmental stages in *Dasyurus*, *Trichosurus*, *Macropus* and *Homo*. From these varied types he concluded that the following fissures were constantly present in all mammals: (1) Fissura Floccularis, (2) Fissura Parafloccularis, (3) Fissura Postnodularis, (4) Fissura Prima and (5) Fissura Secunda.

Other less constant fissures were described, and a common folial pattern for the

mammalian cerebellum was diagrammatically presented. Although recognizing the constant and early development of the fissura floccularis and fissura postnodularis, he failed to use them as landmarks in dividing the cerebellum into the fundamental lobes of his final classification. These lobes he called anterior, middle, posterior and floccular. All the folia anterior to the fissura prima were considered the anterior lobe, those between the fissura prima and fissura secunda, the middle lobe. He called the uvula and nodulus, which are posterior to the fissura secunda, the posterior lobe. He considered the relations of the paraflocculus to the vermician lobules so variable in the different forms studied that he was unable to relate it to any one of the midline divisions. Disregarding the presence of the constant fissure between the flocculus and paraflocculus, he grouped these two parts into a single lobe which he called the floccular lobe.

Bradley (1903, 1904) described the development of the cerebellum in the rabbit and pig and in selected stages in the sheep, calf, horse and the human. In order to divorce himself completely from the use of human anatomical terminology he devised a scheme of letters and numbers to designate the lobes and fissures which he considered fundamental (Fig. 2). To add to the confusion of such a complicated and arbitrary system of nomenclature a close scrutiny of his figures reveals that he has confused the prepyramidal fissure and the fissura secunda in the diagrams of all the cerebella which have a folial pattern simpler than that of the squirrel. In the larger and more complicated cerebella his Fissure III is the one ordinarily known as the prepyramidal fissure, while in the simpler forms the fissure labelled III is apparently what most authors call the fissura secunda. He must be credited with the recognition of the close relationship between the nodulus and flocculus and was not led into the errors of Smith, Bolk and others who placed the flocculus and paraflocculus in a single lobe.

Louis Bolk's work, the result of many years of investigation, appeared in monographic form in 1906. His study was different in method and purpose from that of the others mentioned. He first sought to devise a scheme of cerebellar lobes into which all mammals might be fitted. He visualized the cerebellum as a chain of lobules to which he applied a nomenclature which was for the most part original. It was a combination of names, letters and figures (Fig. 3). Once having established such a common folial pattern, which resembled closely the cerebellum of the adult *Lemur albifrons*, he sought to correlate the size of a particular subdivision with the muscular development in each species. By such comparative studies he suggested a topographical localization in the cerebellum. Bolk's work was the impetus for a large series of ablation experiments by various workers in an effort to test his ideas of localization of function. Many of the terms he applied to the cerebellum have been most useful, because they are readily applicable to all mammals. Riley (1929) presented a similar study which is a convenient reference for the identification of the lobes and fissures in a large series of animals, including many frequently used in the laboratory. Both of these workers, occupied with the differences in the complex folial pattern of adult animals, failed to appreciate the more fundamental divisions of the organ.

Bolk (1906) and Riley (1929, 1930) both divided the cerebellum into anterior and posterior lobes separated by the fissura prima. The posterior lobe was subdivided by Bolk into a lobulus simplex, lying directly behind the fissura prima and a lobulus complicatus comprising the remainder of the posterior lobe. The latter was then subdivided into a medial part, the lobulus medianus posterior, which included the whole of the vermis caudal to the lobulus simplex. A lateral part called

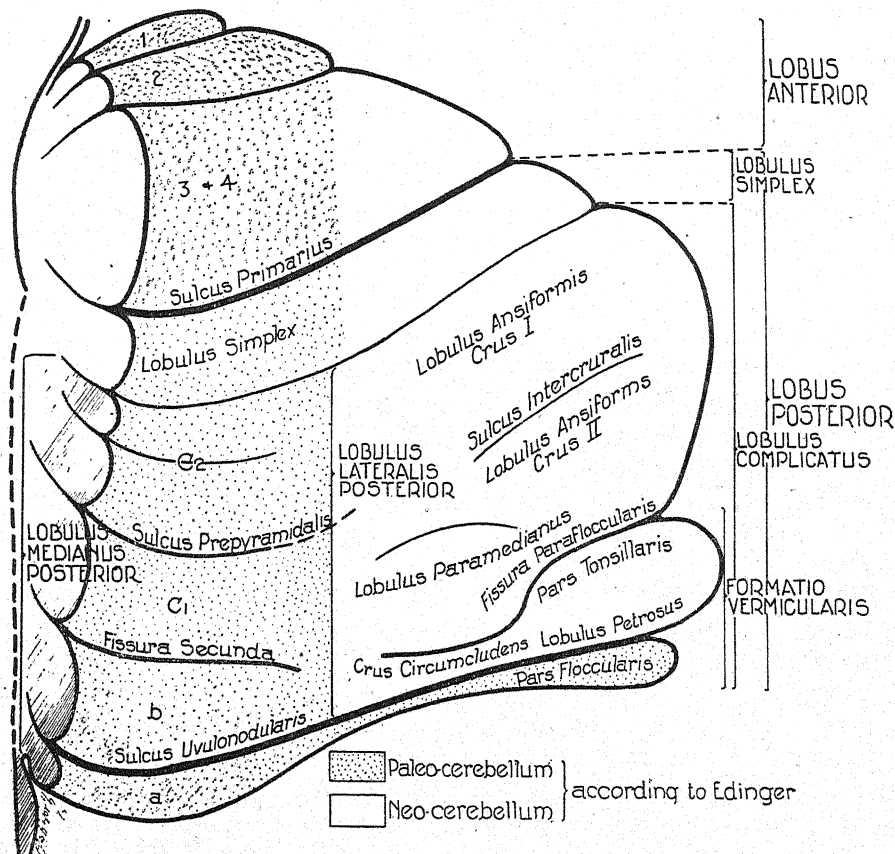


Fig. 3. Schema of the mammalian cerebellum with the lobes named according to the terminology of Bolk (1906). The terminology of Riley (1929) is very similar. The shaded area is that part of the cerebellum considered to be palaeocerebellum and the unshaded portion the neocerebellum as defined by Edinger (1910).

the lobulus lateralis posterior was divided into lobulus ansiformis, lobulus paramedianus, and formatio vermicularis. In the last were included a pars tonsillaris, pars circumcludens, lobulus petrosus and pars floccularis.

Bolk and Riley considered the tonsilla of the human cerebellum to be related to the uvula, and Riley states that the lobulus uvulo-tonsillaris 'may be homologized with the lobulus parafloccularis'. These authors consider the lobulus biventralis, as homologous with the lobulus paramedianus. This lobule is assigned by them to the pyramid of the vermis (Fig. 1).

Observing that the 'hemispheres' are essentially a mammalian structure, Edinger (1910) applied the term 'palaeocerebellum' to the vermis and flocculus and the term 'neocerebellum' to the remainder (Fig. 3). Hausman (1929), elaborating on this concept, stated that a neocerebellar equivalent has been provided for each lobule of the vermis except the lingula. Although useful and expressive, the terms neo- and palaeocerebellum have been used by many different authors to designate somewhat different cerebellar parts, and unless qualified or limited have at present no certain meaning. Further, as Winkler (1923) and others have emphasized repeatedly, new acquisitions to the cerebellum take the form of growth of pre-existing parts rather than altogether new structures superimposed on the old. The identification of the vermis, as defined by Edinger, as a morphological entity is misleading and lacks morphological or functional support. Hausman's identification of all lateral cerebellar parts with the neocerebellum seems hardly justified when we realize that both ontogenetically and phylogenetically the cerebellum had a *bilateral* origin, and that even in man one of the lateral parts, the flocculus, appears very early in the development of the cerebellum. The term neocerebellum has some meaning if reserved for those parts which in the higher mammals have come to be dominated by cortico-pontine connexions; but such a definition allows for no hard and fast delimitation by particular fissures. If the rest of the cerebellum is then designated palaeocerebellum, it must be recognized that it contains subdivisions of varying phylogenetic age.

From a functional point of view probably the most important single contribution to cerebellar morphology is that of Sven Ingvar (1918) (Fig. 4). In his earlier monographic work he combines comparative morphological observations, fibre connexion studies, pathological and physiological investigations in an attempt to subdivide correctly the cerebellum. He was impressed with the uniformity of the lobules anterior to the fissura prima and posterior to the fissura prepyramidalis and with the wide variation in the lobules between these two deep and constant fissures in many species of birds and mammals. He noted the presence of two fissures which appeared to correspond to those in the alligator. A case of cerebellar atrophy which was restricted largely to the lobes lying between the primary and prepyramidal fissures was described, and the distribution of the spino-cerebellar fibres rostral and caudal to these fissures was also pointed out. Using all this evidence, Ingvar divided the cerebellum into three lobes, anterior, middle and posterior.

This division, however, was less illuminating than a second concept which he emphasized later (Ingvar, 1928). This second classification was based on the distribution of afferent fibre connexions. He presented it in a novel way by comparing the cerebellum to a three-story house. The 'basement' consisted of the lobes receiving direct vestibular root fibres, which included the nodulus, uvula, lingula and flocculus. The next story he called the spinal floor. It consists of the lobules anterior to the fissura prima, the pyramis and paraflocculus. The remaining lobes which received predominantly ponto-cerebellar fibres he called the 'cerebral floor'. This last division has in recent years been frequently identified by the term 'neocerebellum'.

Speaking of this division of the cerebellum on the basis of afferent fibre connexions and its comparison to a three-story building, Ingvar stated in 1928: 'This conception does not pretend to be more than a crude schematic one. There undoubtedly exists an overlapping in the cerebellar cortex of the different afferent fibre systems. In spite of its crude schematic character it expresses succinctly, to my mind, not only the evolution of the cerebellar functions, but also the functional localization of the organ.'

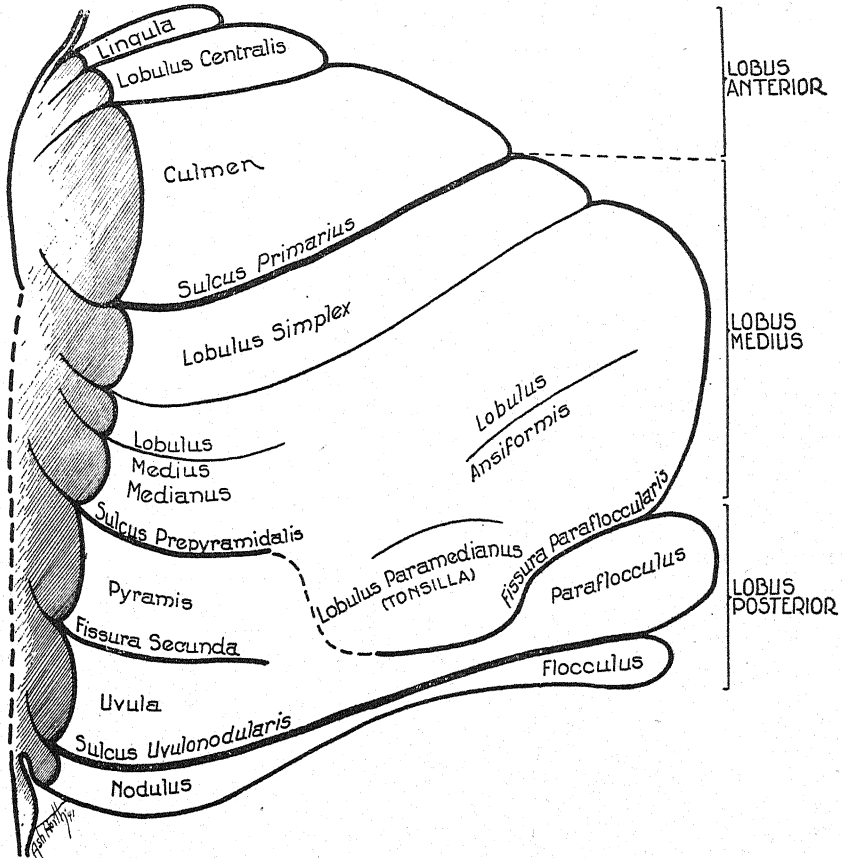


Fig. 4. Schema of the mammalian cerebellum with the lobes named according to the terminology of Ingvar (1918).

Ingvar placed the paraflocculus in the posterior lobe along with the pyramis and uvula. The tonsilla he considered homologous to the lobulus paramedianus of Bolk, and as such a part of the middle lobe which only secondarily comes to lie contiguous to the uvula (Fig. 1). The association of uvula and tonsilla is, according to Ingvar, entirely fortuitous and without morphological significance. He specifically denies that the paraflocculus is homologous to the tonsilla of the human, believing that the accessory flocculus or flocculus secundarii, a vestigial structure, is the human homologue of the paraflocculus.

Elliot Smith (1903*a*) states on p. 379: 'It is misleading to speak of the para-flocculus as "belonging" to any part of the vermis, because the purely mechanical factors of fissure formation may produce all sorts of fortuitous combinations of surface area.' He also points out that this is particularly true in the human. Bolk (1906) considered the tonsilla homologous to a part of the para-flocculus of lower forms, and Riley (1929) considered it a lateral expansion of the uvula and both related the lobulus biventer with the pyramis. Bradley (1904, Pt. II) looked upon the para-flocculus as the lateral expansion of the pyramis and uvula. He states that the tonsilla and lobulus biventer of the human cerebellum arise from that part of the uvula and pyramis, respectively, which in the embryonic development of many subprimate forms served to connect these vermian lobules with the para-flocculus.

More recent morphological studies of the cerebellum have been those devoted to submammalian forms as exemplified by the work of Herrick (1914, 1924), Larsell (1920-32) and others. Following Larsell's wide experience with developmental stages and adult forms in amphibians and reptiles, Larsell & Dow (1935) described the development of the cerebellum in the bat together with selected stages of the mole and rat. Shortly thereafter the development of the cerebellum in the opossum was presented (Larsell, 1935, 1936*a*). The following year, unfortunately too late for inclusion in Kappers *et al.* (1936), appeared a review and interpretation of almost 20 years' work on cerebellar morphology (Larsell, 1937). In this article are defined those divisions which he feels are fundamental in cerebellar morphology and he redefines and crystallizes his conception of the cerebellum built up from a rich background of painstaking study. Larsell differs from all previous investigators by first dividing the cerebellum into the flocculo-nodular lobe and the corpus cerebelli (Fig. 9). The fissure between these two lobes he calls the fissura posterolateralis and states that it is constant throughout the vertebrate series. The other fissures mentioned above and thought by Smith, Bradley, Bolk and Ingvar to represent the fundamental divisions of the cerebellum Larsell would identify as secondary folds within the corpus cerebelli which in mammals is many times larger than the flocculo-nodular lobe. Of these fissures within the corpus cerebelli Larsell feels that the fissura prima should be considered of most importance, and he uses it to divide the corpus cerebelli into an anterior and posterior lobe.

A comparison of Figs. 1, 2, 3, 4 and 9 indicates the various terms applied to the same lobes by the authors quoted above. The greatest difficulty in establishing homologies is in the posterior lobe of the corpus cerebelli. Closely graded developmental stages of many different forms, particularly the higher primates, need to be studied to determine homologies between the lobes of subprimate forms and those of man. A study restricted to adult forms is unsatisfactory. A competent analysis of the development of the cerebellum in several species of primates would be of great importance.

III. PHYLOGENY OF THE CEREBELLUM

(1) *Cyclostomes*

External form. The presence of a cerebellum below *Petromyzon* has been affirmed by many workers (see Jansen, 1930), but more recently Conel (1931) and Jansen (1930) failed to identify positively a cerebellum in the Myxinoidei.

The cerebellum in *Petromyzon* has been recognized for many years. First studied by Robin (1849), many subsequent descriptions have appeared, one of the most recent being that of Pearson (1936). In these forms the cerebellum consists of a bridge of tissue formed by an elevation of the lateral part of the medulla oblongata. It is immediately caudal to the optic tectum, and the root fibres of the trochlear nerve and the cells of the trochlear nucleus are found in the anterior part of the cerebellar plate.

Histology. The histological arrangement of elements suggests forerunners of the granular and Purkinje cells of higher forms. The granular cells are but slightly modified from the small cells of the latero-vestibular area. Their neuraxes are somewhat larger and may cross as commissural fibres in what would be homologous to the molecular layers. Although neither Johnston (1902) nor Tretjakoff (1909) state that true Purkinje cells are present in this form, the large cells of the cerebellar plate are modified slightly as compared with the similar large cells in the latero-vestibular area. Their most striking resemblance to true Purkinje cells is the orientation of their dendrites, which extend up into the primitive molecular layer.

Afferent fibre connexions. Afferent connexions to the cerebellum in *Petromyzon* include direct fibres from the anterior and posterior lateralline nerves and the vestibular nerve, secondary cerebellar fibres from the acoustico-lateral area; primary and secondary trigeminal fibres, spino-cerebellar, tecto-cerebellar and connexions from the hypothalamus. It is obvious that even in this primitive form the cerebellum is already an important centre for the reception of impulses from a wide variety of afferent stimuli. It is on the basis of these connexions even more than the histological structure which led Johnston to identify this part of the brain as the cerebellum.

Efferent fibre connexions and nuclei. The efferent connexions are more simple. Axons of the large cells, forerunners of the Purkinje cells, in common with large cells of the acoustico-lateral area, and the area statica, form the cerebello- and octavo-motor tract. This terminates in the homolateral and ventrolateral motor regions of the medulla oblongata and midbrain.

One of these motor nuclei, the anterior octavo-motor nucleus, has been suggested as a site of migration of cells which form the cerebellar nuclei of higher forms. Its neuraxes terminate in the tegmentum of the opposite side at the level of the oculo-motor nerve. Thus a similarity to the cerebellar nuclei is evident.

(2) *Fish*

No attempt will be made to describe the cerebellum in the wide variations found in various species of fish. For a complete review of this phase of the subject the reader is referred to Kappers *et al.* (1936), from which the following has been summarized.

External form. The cerebellum of the fish has been differentiated into two parts, a median unpaired portion, the corpus cerebelli, and two unpaired portions descriptively called the auricles. The latter are quite uniform in structure throughout the fishes, but the corpus cerebelli shows wide variation. In certain species a lobe of the corpus cerebelli projects forward into the optic ventricle. It is called the valvula cerebelli, and although related to the posterior mesencephalo-cerebellar tract, the function of this specialized lobe is unknown.

Histology. The histological structure of the fish cerebellum shows considerable differentiation. The three typical layers, the molecular, the Purkinje and the granular, all may be identified although the latter is incomplete in places. The molecular layer is more highly differentiated than the others. In many parts of the teleost cerebellum the Purkinje cell layer is several cells thick. Forerunners of both climbing and mossy fibres have been described, although the former are more numerous and more highly differentiated. In the higher teleosts cells resembling the basket cells of the mammalian cerebellum have been described.

Afferent fibre connexions. The fibre connexions include all those described in the cyclostomes. The lateralline and vestibular fibres end predominantly in the postero-lateral part of the organ. Trigemino-cerebellar and spino-cerebellar tracts and tracts from the more cephalad parts of the brain stem end predominantly in the corpus cerebelli. Noteworthy additions include an increase in importance of the spino-cerebellar connexions with the identification of both a ventral and a dorsal spino-cerebellar tract. A definite crossed olivo-cerebellar tract has been identified which is said to end only in the corpus cerebelli. A large number of tracts from more cephalic regions have been described, including tracts from the optic tectum and mesencephalon and a lobo-cerebellar tract from the hypothalamus. These tracts are thought to relay impulses from the optic, static and even olfactory systems to the cerebellum. It has been suggested that certain of these connexions might relay gustatory sensations and impulses originating in the saccus vasculosus which registers changes in fluid pressure.

Efferent fibre connexions and nuclei. The efferent tracts are more complicated than in cyclostomes, but no deep cerebellar nuclei have been identified with certainty.

Tuge (1934, 1935), one of the few to apply degeneration experiments to lower forms, studied Marchi preparations following cerebellar lesions in the teleost. Deep lesions were necessary to produce degeneration in the cerebello-tectal tract. Tuge concludes that this tract does not emerge from Purkinje cells but 'from a deeper layer of the cerebellum'. The remaining efferent connexions, namely, tractus cerebello-tegmentalis mesencephalicus, tractus cerebello-tegmentalis bulbaris,

anterior and posterior and tractus cerebello-acoustico-lateralis, are thought to consist of the axons of Purkinje cells. No direct connexions to nuclei cephalad to the midbrain nor to the spinal cord were found in the fish. Efferent fibres are distributed to the entire motor tegmentum of mesencephalon and medulla.

(3) *Amphibia*

External form. The cerebellum in urodele Amphibia is a more primitive organ than in the fish. In fact it shows few advances beyond the development reached in cyclostomes. The cerebellum is recognizable in all these forms, and its development has been carefully described in both tailed and untailed forms. The amphibian cerebellum furnishes the morphological background for the understanding of the mammalian structure. Its close resemblance to very early embryonic stages in the mammalian cerebellum was emphasized by Herrick in 1914.

The cerebellum of the tailed Amphibia consists of a central unpaired corpus cerebelli and two lateral auricular lobes. In these forms the auricular lobes are well developed, being related primarily to the acoustico-lateral area. In the tailless Amphibia, coincident with the regression of vestibular and lateralline function, the auricular lobes become less well developed.

Development. Larsell (1932a) has furnished a detailed account of the development of the cerebellum in *Amblystoma*. The first indication of the cerebellar anlage is at the early flexure stage (H. st. 32-33). It consists of an accumulation of cells in the dorso-lateral zone of the nerve tube between the cephalic flexure and the Vth roots (Fig. 5). Increase in volume of these areas is followed, in the first day after early swimming, by the growth dorso-medially of the axons of these cells and finally a definite commissural tract is formed. It is along this cerebellar commissure that cells grow medially to form the corpus cerebelli of adult *Amblystoma*. At a distinctly later state, $2\frac{1}{2}$ days after early swimming, a second commissure of fibres is observed passing dorso-medially from the acoustico-lateral area and crossing the midline caudal to the cerebellar commissure. This is called the lateral commissure. Further development in this form consists essentially of enlargement of these commissures by the growth of additional fibres and a proliferation and migration of cells along them. Secondary trigeminal cerebellar fibres, trigeminal root fibres and spino-cerebellar fibres augment the cerebellar commissure. The lateral commissure, consisting largely of unmyelinated fibres from cells of the acoustico-lateral area, receives into it large myelinated VIIIth root fibres.

Histology. The unmyelinated fibres within the commissures are thought to be the forerunners of the parallel fibres of the molecular layer of higher forms. Similar fibres connecting parts of the corpus cerebelli are found in *Amblystoma* and to a greater extent in *Anura*. They originate in small neurons of either the corpus cerebelli or auricle and bifurcate, sending one process medially and the other laterally. The large cells of the corpus cerebelli in adult *Amblystoma* are definitely identifiable as Purkinje cells and the small cells can be said to be granular cells. In the auricular lobe less advanced histogenesis is found. Here the large cells are

thought to be primitive Purkinje cells, and the smaller cells, whose medial processes help to form the lateral commissure, primitive granular cells.

Afferent fibre connexions. The connexions of the cerebellum of *Triturus torosis* will be used as a model of the amphibians in general. This primitive form has been the most instructive of all species studied, according to Larsell (1937), in understanding the morphology of higher forms. It is more generalized than *Amblystoma*,

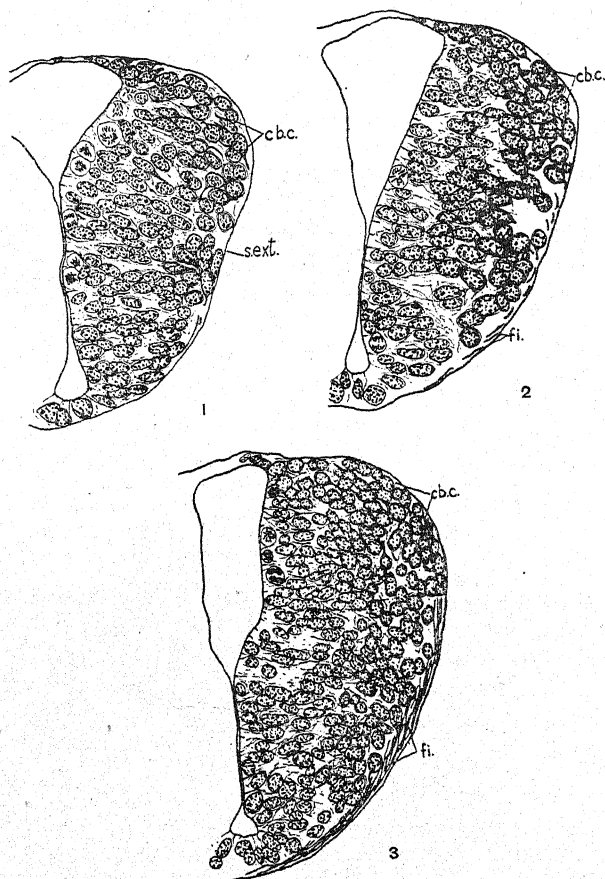


Fig. 5. Cross-sections through cerebellar region of *Amblystoma* larva showing the proliferation of cerebellar cells (*c.b.c.*) and the ingrowth of fibres (*fi.*): (1) early flexure stage; (2) coil stage; (3) early swimming stage. Taken from Larsell (1932*a*, p. 362).

yet contains all the features of the amphibian cerebellum. As compared with *Necturus*, development has gone much further, so that a comparison can be made with the cerebelli of higher forms.

The most important afferent connexion to the cerebellum of the *Triturus torosis* (Larsell, 1931) is the ascending root of the VIIIth nerve. It proceeds dorsal to the entering Vth roots and the trigeminal parts of the cerebellar commissure to enter the auricle from a ventral direction. It arches dorsally around the grey cellular mass

connecting the auricles with the corpus cerebelli and tegmentum. It terminates homolaterally in the whole of the auricle and becomes incorporated in the mass of fine fibres having their origin in the small cells of the auricles. These two components constitute the lateral commissure first clearly seen in this form. This commissure and the interconnexions which it establishes between the two auricles is so well developed that a surface marking may be seen separating this part of the

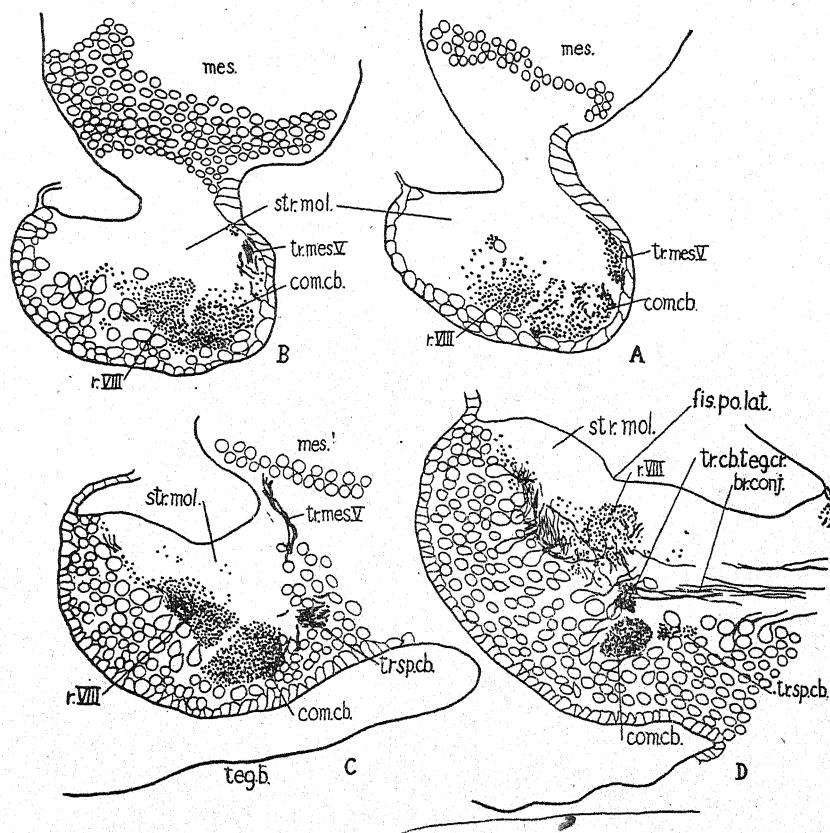


Fig. 6. Sagittal and parasagittal sections through the cerebellum of *Triturus torosus*. A, mid-sagittal; B, C and D represent parasagittal sections. Note the division of the fibres into two commissures, one about the eighth root (r. VIII), the lateral commissure, and a more rostral cerebellar commissure (com.cb.). In the most lateral section D, an indication of the postero-lateral fissure is seen (fis.po.lat.). Modified from Larsell (1931, p. 8).

cerebellum from the corpus cerebelli. This transverse groove on either side is the forerunner of what Larsell believes to be the most fundamental boundary in cerebellar morphology, the postero-lateral fissure (Fig. 6).

The cells of the auricle, although receiving mainly vestibular and lateralline connexions, extend their dendrites into the adjacent parts of the corpus cerebelli which as we shall see receive impulses from many other sources. Other connexions to the cerebellum end predominantly in the corpus cerebelli and help to make up

the cerebellar commissure (Fig. 7). The cerebellar commissure, located rostral-ventral to the lateral commissure, is made up for the most part of coarser, myelinated fibres. They are spino- and bulbo-cerebellar fibres from the spinal cord and medulla, direct Vth root fibres, and axons of cells lying in the ventro-lateral part of the corpus cerebelli and in the region of the transition between this and the anterior Vth nucleus. These cells are connected by collaterals with fibres of the Vth root and the mesencephalic Vth root. Although the direct and secondary Vth root fibres are in part intertrigeminal connexions ending in the opposite Vth nuclei and tegmentum, some terminate in relation with the cells of the body of the cerebellum. The spino-cerebellar fibres, including bulbo-cerebellar fibres, all terminate in the corpus cerebelli. In *Amblystoma*, Larsell (1920) originally felt these fibres could be separated into a dorsal and ventral bundle. He later concluded that only the ventral exists here

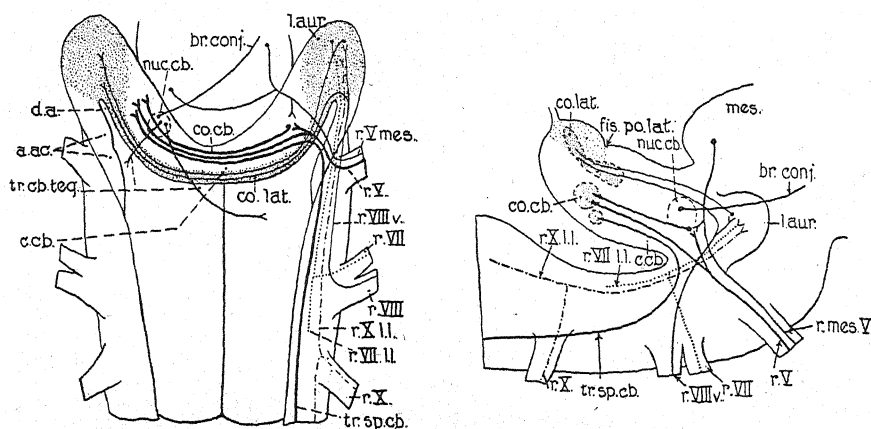


Fig. 7. Diagrams of the amphibian cerebellum showing fibre connexions. Taken from Larsell (1937, Fig. 1 A, B).

and that which he had called the dorsal spino-cerebellar tract in *Amblystoma* was part of the ascending Vth bundle.

Although not definitely certain of their origin, Larsell (1931) found scattered coarse fibres from the subthalamic region to the corpus cerebelli. They were called the mammiilo-cerebellar tract. Tecto-cerebellar fibres originating in *Triturus* from small cells in the caudal pole of the tectum likewise terminate in the body of the cerebellum.

Efferent fibre connexions and nuclei. There are crossed and homolateral efferent connexions from a mass of cells in the rostral part of the corpus cerebelli where it is continuous with laterally placed auricles. It is distinct enough in the frog to be designated the nucleus cerebelli. The fibres pass to the midbrain to terminate in that part of the tegmentum in which in the higher forms the nucleus ruber makes its appearance. Fasciculi from the auricles may enter into this bundle. Whether or not axons of Purkinje cells help to form this tract could not be determined with certainty, although Larsell states there are some indications that they do so. In addition to this tract, efferent connexions with rostral and caudal levels are established by tracts

from the acoustico-lateral areas. Into these bundles are incorporated axons of cells in the auricles. These in Amphibia are identified as the tracts *a* and *b* of Kingsbury (1895). Larsell concludes that tract *a* is primarily a correlating tract between various levels of the acoustico-lateral area and contains many functional components, among them what probably correspond to cerebello-vestibular connexions of higher forms. The latter, tract *b*, is largely ascending and probably does not include any efferent cerebellar connexions.

(4) Reptiles

External form. The cerebellum in reptiles varies greatly from species to species. In certain lizards and snakes it consists of a small midline plate of nervous tissue hardly larger than that of *Petromyzon*. The histological structure and fibre connexions are more complex, however. In larger reptiles in which appendages are well

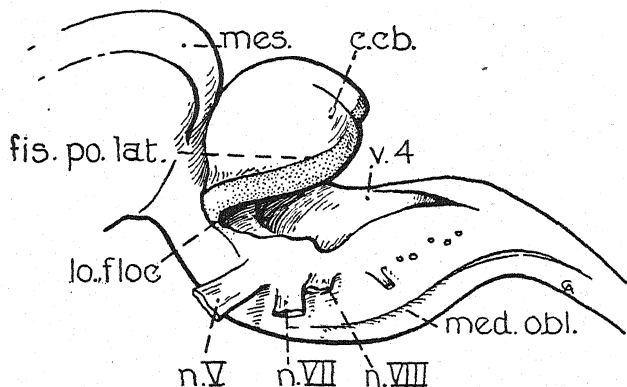


Fig. 8. Diagram of cerebellum of *Alligator mississippiensis*, Reese's stage XXI+. Modified from Larsell (1934, Fig. 6).

developed the cerebellum is increased in size, particularly in its lateral part. Larsell (1926, 1932*b*) has suggested that the development of the lateral part is associated with the use of the appendages in those species in which it is well developed. He suggests that this may be the forerunner of the development of the cerebellar hemispheres in the mammals.

Development. By studying the development of the cerebellum in turtles and particularly in the alligator, Larsell (1932*b*) was able to identify in these forms the same two fundamental divisions as described in Amphibia. The cerebellum of an alligator embryo at Reese's stage XXI+ shows these two divisions particularly well (Fig. 8). Later stages of development show the appearance of other fissures. These are the ones which Ingvar (1918) identified in the alligator and which he considered homologous to fissures to which he gave primary position, the fissura prima and fissura praepyramidalis. Larsell (1932*b*) considers them as secondary folds in the corpus cerebelli, which in reptiles, birds and mammals becomes more and more massive. Although the posterior of these two fissures in young and adult specimens appears to extend into the floccular sulcus, this is a secondary relationship, as the earlier developmental stage clearly shows (Fig. 8).

Along with the development of the corpus cerebelli there is a medial growth of cells and fibres along the commissure of fibres between the two auricular lobes (flocculi). This is the first appearance of the homologue of the mammalian nodulus and thus of the flocculo-nodular lobe complete.

Histology. All the essential elements of the cerebellar cortex as seen in mammals, namely, the granular cells with their unmyelinated axons, the parallel fibres, Purkinje cells and basket cells, are readily identified in reptiles (P. Ramón y Cajal, 1891, 1894, 1896; Larsell, 1932*b*). Considerable differences exist between various species, the alligators showing the most complicated and highly differentiated histological structure. The basket cells are quite primitive. The Purkinje cells have less elaborately branching dendrites than many teleosts, birds and mammals. They show more complexity than do those of the sharks or amphibians, but have very few collaterals as compared with birds and mammals.

Afferent fibre connexions. Afferent fibre connexions include dorsal and ventral spino-cerebellar tracts. These tracts are noted by Larsell to end in greatest number in the anterior and posterior parts of the corpus cerebelli. The vestibulo-cerebellar fibres, both direct and secondary, end chiefly in the medial cerebellar nucleus and in the homolateral and contralateral flocculi (auricular lobes). This distribution suggests the lateral commissure of the amphibian cerebellum. Additional afferent connexions include an olivo-cerebellar tract, a tecto-cerebellar tract and direct trigeminal fibres and a trigemino-cerebellar tract. The Vth nerve connexions are said to end in the corpus cerebelli, while no specific statement is made relative to the points of the cerebellum to which the olivo- and tecto-cerebellar fibres pass.

Efferent fibre connexions and nuclei. Medial and lateral deep cerebellar nuclei have been identified by all workers in the reptiles. The cells of the medial nucleus send their axons to the reticular formation of the brain stem on both sides, caudal and rostral to the cerebellum and to the vestibular nuclei. This corresponds with the connexions of the fastigial nucleus as determined experimentally in mammals (Allen, 1924). The medial nucleus is closely associated with the vestibular nuclei as is also the case in mammals.

The lateral nucleus, although clearly separate dorsally, is continuous with the medial one ventrally. It is thought by Larsell to be homologous to the intermediate nucleus of mammals, from which, in the late stages of the development of the mammalian cerebellum, the dentate nucleus is derived by further differentiation (Dowd, 1929).

The axons of the lateral nucleus are directed almost exclusively rostrally and constitute the cerebello-rubral tract of these forms. A few fibres are said to end more diffusely in the bulbar tegmentum. Larsell states that the efferent fibres arise apparently exclusively from the deep nuclei. Weston (1936), however, believes that the axons of Purkinje cells may contribute to these tracts without relay in the deep nuclei. Because degeneration experiments have not as yet been applied to this problem in reptiles, no positive position may be taken. However, it has been demonstrated that there are such direct fibres from the phylogenetically older parts

of the mammalian cerebellum, and it is reasonable to expect that they would likewise be present in the reptiles.

(5) *Birds*

The cerebellum of birds will be treated with extreme brevity. No detailed study of the cerebellar development in avian forms has appeared since the illuminating work on the amphibian brain. Without the light which these studies would throw on such a subject, it is difficult to fit the investigations of Shimazono (1912), Brouwer (1913*a*) and Ingvar (1918) on this subject into the evolutionary scheme herein presented. This is also difficult because of the divergence of avian forms from the evolutionary tree.

In the avian cerebellum an increase in the relative size of the corpus cerebelli is noted. This goes hand in hand with the increasing importance of the spino-cerebellar fibre connexions. These connexions are, as in reptiles and mammals, chiefly confined to the anterior and posterior parts of the corpus cerebelli, leaving the middle folia and flocculus free of direct spino-cerebellar fibre connexions. The cerebellar cortex shows increase in complexity of the individual elements, particularly in the increase in number of granular cells. The architecture of the avian cortex corresponds in every respect to that of mammals. The other fibre connexions are not essentially different from those described in reptiles. Degeneration experiments have served to supplement the observation of normal material in some instances. Kappers *et al.* (1936) call particular attention to the preponderance of connexions from the spinal cord in birds and the relatively few connexions from rostral centres. This relative predominance of spino-cerebellar fibres is more pronounced than at either end of the phylogenetic scale, for in fishes there are important tectal and hypothalamic cerebellar tracts, and in mammals there are important cortico-ponto-cerebellar connexions.

The homologies of the cerebellar nuclei of birds is difficult in the light of present knowledge. A combination of careful developmental studies in avian forms plus degeneration experiments will be necessary to establish them. A medial nucleus corresponding to the same nucleus in reptiles and mammals is undoubtedly present. Its connexions are similar to those described in reptiles. The more laterally located cells have been divided by Ramón y Cajal (1908) into three separate nuclei, but Kappers *et al.* (1936) were unable to come to definite conclusions. For a more complete discussion of this problem the reader is referred to Kappers *et al.*

Efferent connexions are established with the vestibular nuclei, the reticular formation of the bulb and tegmentum, to the red nucleus and even to the diencephalic centres. As in lower forms, connexions to rostral nuclei come from the more lateral of the deep cerebellar nuclei. Some evidence is found for direct connexions from parts of the cerebellar cortex to the nuclei of the vestibular region and bulbar tegmentum.

IV. ANATOMY OF THE MAMMALIAN CEREBELLUM

(1) *External form*

The present review will not concern itself with a detailed discussion of the external form of the mammalian cerebellum in various species.

No terminology thus far applied to the mammalian cerebellum is entirely satisfactory. One which can best be applied to the whole vertebrate series corresponds most closely to that of Larsell. It must be emphasized, however, that sharp boun-

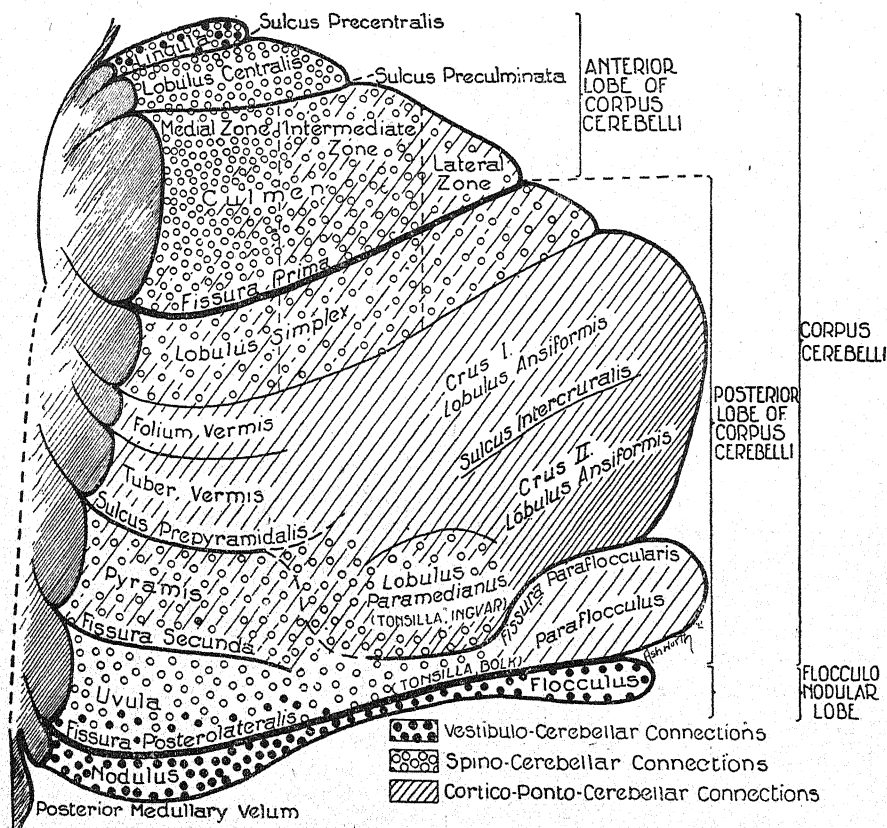


Fig. 9. Schema of the cerebellum. The brackets on the right show the divisions according to Larsell's classification. Afferent fibre connexions as determined by oscillographic studies are indicated by the different types of shading.

daries of functional importance may not exist in the cerebellum, and if they do may not necessarily correspond with the classification of lobes and fissures herein presented. As our goal should be a nomenclature having functional as well as morphological significance, subdivisions of the larger lobes must be included to allow for systematic functional studies of various parts. Fig. 9 presents a schema of the mammalian cerebellum which incorporates the terms which seem to the author best suited to the cerebellum of all vertebrates. It follows very closely the

terminology presented by Larsell in 1937, since adopted by Fulton (1938) and others.

Neo- and palaeocerebellum. The outstanding change from lower forms is the expansion of the lateral parts of the corpus cerebelli. In primates and particularly in anthropoid apes and man, the so-called hemispheres reach their greatest size and complexity. This lateral expansion develops coincidentally with and probably under the influence of a new afferent connexion to the cerebellum, namely, the cortico-ponto-cerebellar connexions. These are exclusively mammalian, and the lobes in which these fibres end in greatest number are rightfully called the 'neocerebellum'. Inasmuch as there are apparently a considerable number of pontine fibres which end in the central lobes of the vermis, particularly the upper parts of the culmen, lobulus simplex and the folium and tuber vermis, the use of the term 'neocerebellum' as synonymous with the hemispheres and the palaeocerebellum to include the whole of the vermis and flocculus, is not believed to be in accord with the facts of comparative neurology. Unfortunately, it was in this sense that the terms neo- and palaeocerebellum were originally applied by Edinger in 1910.

Homologies of the human tonsilla and lobuli quadrangularis. Particular attention should be directed to the position of the lateral parts of the posterior lobe, long a controversial issue. In man the terminal part of the lateral outgrowth of uvula and pyramis is vestigial. This part, so prominent in some mammals, is rightly termed the paraflocculus and is homologous to the larger lobe of the same name in lower mammals. However, it seems unjustified in the light of present knowledge to force a homology between the tonsilla of the human cerebellum and the lobulus paramedianus, as Ingvar has insisted. One is tempted to agree with Bradley (1903, 1904) that the tonsilla of the human is not homologous with either the lobulus paramedianus or the paraflocculus. Possibly it is a growth, peculiar to anthropoid apes and man, of the stalk of the paraflocculus which in most mammals connects the paraflocculus with the uvula and pyramis. A final decision cannot be reached until its development in the higher primates can be studied and its fibre connexions in the human determined.

The lateral expansion of the culmen and lobulus simplex, so large in higher primates, might be given a separate name. It is recognized that these lobes expand in these higher forms particularly under the influence of important cortico-ponto-cerebellar connexions. Their connexions with the inferior olive (Brodal, 1940a) indicate a division in a median, intermediate and lateral zone. The most lateral zone apparently is lacking in some species, including the cat. For description of the folial pattern of a large series of mammals, the reader is referred to the work of Bolk (1906) and Riley (1929). A complete description of the folial pattern of the cerebellum of the rabbit may be found in the recent paper by Brodal (1940c). A recent valuable discussion of the homologies between the human and lower forms is to be found in Brodal (1940a).

The cerebellar peduncles. With the increase in size of the cerebellum in mammals and particularly the development of massive fibre tracts leading to and from the organ, definite peduncles are present. These are commonly known as the inferior,

middle and superior cerebellar peduncles. The inferior peduncle has been divided into a lateral fibre bundle, the restiform body, and an inner part of mixed fibres and cells. The inner part is known by various names and is most commonly called the juxta-restiform body. It is continuous with the medial and intermediate nuclei above and the vestibular nuclei below. The fibres consist of cerebello-vestibular and vestibulo-cerebellar connexions. The terms brachium pontis and brachium conjunctivum are used synonymously with middle and superior peduncle, respectively.

(2) *Development as exemplified by the opossum*

The only studies of the development of the mammalian cerebellum in the light of the recent findings in lower forms have been those of Larsell & Dow (1935) and Larsell (1935, 1936*a*). Closely graded stages of the opossum, beginning with the earliest histological evidences of the cerebellum, have been described. The development of the external form and fissures in the opossum, bat, rat and mole has been followed. As stated above, similar studies of higher mammalian forms, particularly primate material, is needed, and a re-examination of human material might be a fruitful investigation. The development described below will be taken from the description by Larsell (1935, 1936*a*) of the development in the opossum.

The first indication of the cerebellum in the opossum appears in the eleventh day in McCrady's stage 30 (McCrady, 1938). There is first a growth of commissural fibres medially across the midline. In addition to the commissures the anlage of the cerebellum consists at this stage of a layer of cells, undifferentiated from their fellows in the hindbrain and midbrain, which is continuous rostrally with the midbrain and caudally with the tela chorioidea. The commissural fibres, just as in Amphibia, are separated into two groups. The more rostral, coming from the Vth root, is called the commissura cerebelli, and the other located more caudally and consisting of VIIIth root fibres, the commissura lateralis. At the same time as these two commissures form, a proliferation of cells occurs lateral to the midplane in the areas through which the commissural fibres pass on their way to the midline cerebellar arch.

In McCrady's stage 31 (late 11th day) there appears, first laterally, a shallow groove parallel to the lateral commissure. This groove eventually fuses in the midline and becomes the postero-lateral fissure which had been identified as the earliest fissure to form in the bat by Larsell & Dow (1935).

By stage 33 of McCrady (late 12th day) there is a bilaterally symmetrical, massive corpus cerebelli and a flocculo-nodular lobe. The migration of cells medially along the cerebellar commissure precedes a similar migration along the lateral commissure. This earlier development of the corpus cerebelli in the midsagittal plane can also be seen in the 13 mm. embryo of the bat (Fig. 9, Larsell & Dow, 1935). Further development consists of enlargement of the cerebellar commissure by increasing number of primary and secondary trigeminal connexions and spino-cerebellar fibres. The cells of the tegmentum grow up into the base of the corpus cerebelli, first as a single mass of cells and later developing into three parts distinguishable in the adult as the nuclei fastigii, interpositus and dentatus. In the

opossum these nuclei never become completely separate from the cells of the tegmentum, being continuous both with the tegmental region and the superior vestibular nuclei. The ponto-cerebellar fibres, which in higher mammals assume such a conspicuous role, grow in laterally on either side without entering intimately with either the medulla or basal cerebellar nuclei.

The growth of the corpus cerebelli far outstrips that of the flocculo-nodular lobe. Many fissures develop in this part of the cerebellum even in this primitive mammal. The first one to form is the so-called fissura prima, though it is preceded by the postero-lateral by 10 days. This fissure is seen first in the 8-day pouch young (18 mm. C.R. length). Its appearance at a significantly later stage than the postero-lateral fissure is in confirmation of Larsell & Dow's (1935) observation in the bat and is opposed to Abbie's (1934) contention that the fissura prima was the first to form.

The fissures which subdivide the corpus cerebelli appear in the following order: (1) fissura prima, (2) fissura secunda, (3) fissura preculminata, (4) fissura para-floccularis, (5) fissura prepyramidalis, and (6) fissura intercruralis.

The developmental studies cited above have pointed out, with particular emphasis, the morphological distinction between the flocculus and para-flocculus. These two parts of the mammalian cerebellum are separated by the most fundamental landmark of cerebellar morphology, the postero-lateral fissure. One, a part of the flocculo-nodular lobe, is foreshadowed even in aquatic forms, as the auricular lobe, whereas the para-flocculus is foreshadowed in birds and reaches full development only in mammals. In mammals it appears at a much later ontogenetic developmental stage than does the flocculus.

(3) *Histology*

The extensive cytoarchitectonic studies of the cerebral cortex have had no counterpart in the cerebellum. Indeed, it is generally agreed that there are no significant histological differences in the various lobes of the cerebellum.

Purkinje cells. The fully developed cortex is divided into an outer molecular and an inner granular layer by a row of large pear-shaped cells, the Purkinje cells. These cells are about $35-50\mu$ in diameter in man. The dendritic branches, which are exceedingly complex, extend from the peripheral part of the cell body to the surface of the cerebellum but never into the granular layer. Instead of branching in all directions they are flattened laterally so that their extent is seen only in sections, cut at right angles to the cerebellar folia. In sections parallel to the folia the dendritic tree is viewed on edge so to speak. The axon is given off from the deeper pole of the cell. It is the efferent pathway from the cortex to the deep cerebellar nuclei. These axons have collaterals which return to the adjacent molecular layers and possibly are responsible for the association fibres described by Jansen (1933), although no definite statement can be made on this point.

Granular cells. Other elements of great importance are the granular cells with their processes. These are very numerous small cells lying between the Purkinje layer and the white matter of each folium. They have a scanty cytoplasm and give

off six or seven short dendrites which end in heavy claw-like processes which synapse with the terminations of the afferent fibres, known as mossy fibres. The mossy terminals of the incoming fibres, together with the dendrite claws of the granular cells, form protoplasmic islands or tiny glomeruli. Each incoming fibre may divide into twenty or thirty terminal branches and supply a considerable area of a single folium or even more than one folium and thus maintain synaptic connexion with many scattered granular cells. The axon of the granular cells is a fine unmyelinated fibre which extends toward the surface until it reaches the molecular layer. Here each forms a T- or Y-shaped division sending a process in either direction parallel with the outline of the folium and at right angles to the dendritic spread of the Purkinje cells. These axons apparently make synaptic connexions with the dendrites of the Purkinje cells in the path to which they are related. Bodian (1940) has been able to show that the spiny processes, also called 'thorns', on the dendrites of Purkinje cells, so prominent in Golgi's preparations, are in all probability the small end-feet from the axons of these granular cells.

Basket cells. A third cellular element which apparently also serves to diffuse the incoming impulse to many Purkinje cells is the so-called stellate or basket cell. The cell bodies of these neurons are found in the molecular layer. They are about one-third the size of the Purkinje cells. Their dendrites extend throughout the molecular layer oriented similarly to the Purkinje cells, but less regularly so. The neurons extend horizontally and transversely, giving off ascending collaterals into the molecular layer where they synapse with Purkinje cell dendrites (Estable, 1923) and descending collaterals which form pericellular basket-like endings about a series of adjacent Purkinje cell bodies and the proximal unmyelinated part of the Purkinje cell axon. The stellate cells, located near the surface, may not form typical basket-like endings, and every gradation may be found between the two types. These dendrites are thought to be synaptically related to the axons of granular cells.

Scattered among the granular cells are the cells of Golgi. These are large, multipolar neurons whose extensive branching dendrites extend in all directions in both the molecular and granular layers. The axonic processes after a short course break up into a multitude of small terminal branches. These are thought to be in synaptic relation with dendritic processes of many granular cells. Other scattered cells have been described and are discussed in detail by Ramón y Cajal (1909-11).

Mossy and climbing fibres. The incoming impulses to the cerebellar cortex are generally regarded as carried by fibres of two types, the mossy fibres and climbing fibres. Lorente de Nó (1924) and Snider (1936), however, consider the climbing fibres more likely to be the termination of association fibres. The mossy fibres synapse with the dendrites of the granular cells. The climbing fibres form synaptic connexion with the dendrites of the Purkinje cells in the molecular layer. These terminate apparently about a single Purkinje cell. Thus we find in the cerebellum two types of afferent connexions, one seemingly designed to diffuse an incoming impulse widely over a considerable area of the cerebellar cortex; the other to establish a highly specific synaptic connexion to a particular cell which in turn sends its axon to a more or less specific part of the deep nuclear system (Jansen & Brodal,

1940). The origin of the climbing and mossy fibres is not clear and will be discussed later.

(4) *Afferent fibre connexions*

The afferent connexions to the cerebellum come from four principal sources: (1) the vestibular nerve and vestibular nuclei; (2) the spino-cerebellar tracts, dorsal and ventral, together with fibres from the external cuneate nucleus, and the related fibres from the reticular formation and trigeminal nuclei; (3) the ponto-cerebellar fibres which relay impulses from the cerebral cortex; and (4) the olivo-cerebellar fibres from the inferior olivary nuclei. Each of these systems has certain distinctive features in the mammalian cerebellum which should be emphasized. The study of the action potentials of the cerebellum in response to stimulation of their afferent systems has added to our knowledge of these connexions (Fig. 9).

Vestibulo-cerebellar connexions. Vestibulo-cerebellar connexions consist of direct root fibres and vestibular nuclear connexions. The exact cells of origin for these secondary vestibulo-cerebellar fibres are not known, but it has been generally assumed that they arise in the superior nucleus of Bechterew and the lateral nucleus of Deiters (Larsell, 1939). Vestibular root fibres to the cerebellum have been described in detail by Ingvar (1918) and Dow (1936). They terminate in the cat and rat in the homolateral flocculus, the homolateral half of the nodulus and uvula and lingula (Ingvar), as well as the fastigial nucleus. The fibres to the vermal lobes enter through the juxta-restiform body after traversing the lateral and superior vestibular nuclei. The vestibular root fibres to the flocculus pass into the cerebellum in the cat lateral to the restiform body proper (Dow, 1936). Secondary vestibulo-cerebellar fibres were found distributed bilaterally to the identical parts, including the lingula, following a lesion of the juxta-restiform body in the rat (Dow, 1936). Single-shock electric stimulation of the VIIIth nerve in the cat (Dow, 1939) resulted in cerebellar action potentials in both flocculi, the nodulus, uvula, lingula and the fastigial nuclei, but only in these parts.

Spino-cerebellar connexions. Spino-cerebellar connexions consist of the well-known dorsal and ventral spino-cerebellar tracts (MacNalty & Horsley, 1909; Ingvar, 1918; Beck, 1927; Jansen, 1931) and connexions by way of the external cuneate nucleus and related nuclei of the restiform body. The ventral spino-cerebellar tract enters the cerebellum by passing over efferent fibres of the superior cerebellar peduncle and terminates in the anterior lobe exclusively. The dorsal spino-cerebellar tract and fibres from the external cuneate nucleus help to make up the restiform body. Various workers have described cerebello-petal fibres from the nuclei gracilis and cuneatus proper (Mussen, 1927), and such connexions are regularly given in textbooks (see Kappers *et al.* 1936). The recent work of Ferraro & Barrera (1935) indicates, as Winkler showed in 1918, that the fibres to the cerebellum come only from the external part of the cuneate nucleus, the remainder of the nuclei of the dorsal columns being connected solely with the thalamus. Winkler (1918) and Allen (personal communication) also showed that all the large cells of the nuclei gracilis and cuneatus proper degenerate after section of the medial lemniscus. In a single

chimpanzee, Walker (1938*b*) likewise found fibres to the cerebellum only from the lateral part of the cuneate nucleus. Brodal (1941) has determined by degeneration methods that the fibres from the external cuneate nucleus terminate in the same lobes of the cerebellum as do the dorsal and ventral spino-cerebellar fibres. Regardless of the route by which the impulses take to reach the cerebellum, they eventually terminate in the cat, as shown by oscillographic methods (Dow, 1939), in the entire anterior lobe of the corpus cerebelli, in the lobulus simplex, pyramis and lobulus paramedianus. The responses were most marked homolaterally, but slight responses were found on the contralateral side as well. As estimated by the strength of the action potential response, the endings were most numerous in the anterior lobe. The same lobes showed the action potential regardless of whether the nerves of the upper or lower extremity were stimulated or if the spino-cerebellar fibres were stimulated directly in the spinal cord. Indeed, responses of the same type and same distribution were seen following stimulation of the dorsal reticular formation and in a few experiments, elsewhere unreported, following stimulation of the trigeminal nerve. The route by which the trigeminal fibres reach the cerebellum is not known. Long suspected on morphological grounds there has been little experimental evidence of their existence in mammals. The lobes of termination and lack of topographical localization within the spino-cerebellar part of the cerebellum is in general agreement with the findings of McNalty & Horsley (1909), Ingvar (1918), Beck (1927), and Jansen (1931). Brodal & Jansen (1941) have recently studied by Marchi technique the termination of the dorsal and ventral spino-cerebellar tracts in a woman five weeks after bilateral chordotomy. The lobes of termination are predominantly the middle and intermediate zones of the anterior lobe, especially the lobulus centralis and lower part of the culmen. Some fibres go to the pyramis, and occasional isolated fibres to the middle lobe of the vermis, uvula and nodulus. Reticulo-cerebellar fibres have been described by Van Gehuchten (1902, 1904), Molhant (1910) and Papez (1930). They enter the cerebellum by way of the inferior cerebellar peduncle.

Cortico-ponto-cerebellar connexions. The details of the cortico-ponto-cerebellar connexions, important though they are in mammals, are not well known. Sunderland (1940) has recently published an excellent review of the literature on this subject together with important original findings in monkeys.

Cortico-pontine connexions. Although it is generally agreed that the frontal lobe is an important source for cortico-pontine fibres, the exact cytoarchitectonic areas responsible for the fronto-pontine fibres are still in dispute among students of the subject. Mettler (1935*b*) states that pontine fibres arise from area 9 but not from areas 4 or 6. Levin (1936) describes in the monkey pontine fibres from areas 4 and 6, and states that fibres occupying the medial part of the cerebral peduncle (Arnold's bundle) come from more rostral areas, possibly the inferior frontal gyrus. Sunderland (1940) denies the presence of a pontine projection from area 8 and states that it is unlikely that any exist from areas 9 and 10. He finds area 6 contributing to the medial cortico-pontine tract. In this he is confirmed by Verhaart & Kennard (1940) who studied the degeneration following small lesions confined to area 6, area 4-strip

of Hines (1936), and area 4. Degeneration which terminated in the pons was found following a lesion in area 6. It occupied the medial one-third of the pes pedunculi. Areas 4s and 4 likewise send cortico-pontine fibres which occupy the intermediate parts of the internal capsule and pes pedunculi along with the cortico-spinal fibres.

Recent studies concerning the temporo-pontine fibres are those of Mettler (1935*d*), who described such fibres from all three temporal convolutions, of Rundles & Papez (1938) who found no degeneration in the outer part of the cerebral peduncle following temporal lobectomy in monkeys and baboons, and Sunderland (1940) who described extensive degeneration following isolated lesions of the cortex of the temporal lobe. Important parietal pontine projections occupying also the lateral part of the peduncle have been found by all recent workers, including Biedmond (1930), Clark & Boggon (1935), Mettler (1935*c*), Rundles & Papez (1938) and Sunderland (1940). Occipito-pontine fibres, though apparently less numerous than temporo- and parietal components, have been described by Sunderland (1940) confirming the conclusions of Poliak (1927), Mettler (1935*a*) and many other earlier workers. Concerning the termination of these various systems in the various parts of the pons, even more confusion exists. The limitations of the Marchi method make conclusions almost impossible. Sunderland concludes from his own observations that: 'In the pons all the cortico-pontine fibres revealed by this study terminate ipsilaterally, in approximately the rostral three-fourths of the pons. The fronto-pontine fibres appear to terminate about the dorsal part of the pontine nucleus chiefly, while all the others end in relationship with the ventrolateral and lateral portions of the nucleus. The frontal fibres terminate more rostrally, and the parietal more distally than the remainder, though there is a considerable overlap of all systems in the intermediate zones.'

Ponto-cerebellar connexions. Ponto-cerebellar fibres were said to be distributed to the hemispheres only, according to Thomas (1912) and Masuda (1914). Fibres end in the vermis also, according to Spitzer & Karplus (1907), Besta (1912), Winkler (1927), Jakob (1928) and Sinnige (1938). All workers are agreed that they reach the cerebellum by way of the middle cerebellar peduncle. Vejas (1885), Borowiecki (1911), Masuda (1914), Uemura (1917) and Brun (1925) consider that only a contralateral connexion exists. Lewandowsky (1904), Marburg (1922), Winkler (1927), Sinnige (1938), Sunderland (1940) and others consider the relationship predominantly crossed, but believe some homolateral connexions are present as well. In a few experiments the middle peduncle at its entrance into the cerebellum was damaged inadvertently in connexion with other work (Dow, 1936). Degenerated fibres of undoubted pontine origin could be traced not only to the hemisphere on the side of the lesion but to the vermis and to the opposite lobulus ansiformis and paraflocculus. Such fibres must be considered when retrograde cellular changes are studied in the pons following deep ablations of the cerebellar lobes.

It is evident from oscillographic studies (Curtis, 1940) that action potentials smaller than those on the contralateral side may be found on the homolateral cerebellar cortex in cats. The author has confirmed this in cats and monkeys (Dow,

1942), but no information is available as to how these homolateral connexions are made.

Electrical stimulation of the pons results in cerebellar action potentials in the cat in the lobulus ansiformis, paraflocculus, lobulus paramedianus, folium and tuber vermis, lobulus simplex, pyramis and upper part of the culmen (Dow, 1939). Curtis (1940) found action potentials in the lobulus ansiformis, lobulus paramedianus, lobulus simplex and upper part of the culmen following stimulation of the cerebral cortex in the cat. In my experience in recent work, in both cats and monkeys (Dow, 1942), responses on cerebral cortical stimulation have been found in all lobes from which we had obtained potentials following stimulation of the pons. Sunderland (1940) found diffuse degeneration of the pontine nuclei most marked contralaterally following ablations of the lobulus simplex, lobulus ansiformis and lobulus paramedianus, but not following a lesion of the lateral part of the culmen. No lesions restricted to the vermis or paraflocculus were studied. Because of the diffuseness of the changes observed, Sunderland (1940) concluded: 'The rostral half of the pontine nucleus projects chiefly on the contralateral lobus medius (Ingvar) of the cerebellum. There is no strict projectional localization in the sense that a particular part of the nucleus projects exclusively to any specific cerebellar area. All aspects of the nucleus appear to project diffusely over the surface of the lobus medius.'

Brodal (1940*b*), in work as yet incompletely reported, however, has been able to show changes in specific parts of the pons, following lesions of the paraflocculus, in new-born animals. It is possible that, by using methods described by Brodal (1939, 1940*b*), more exact information concerning ponto-cerebellar connexions may be obtained. One awaits with interest a complete report of this work. Masuda (1914) and Winkler (1927) felt that there was a predominance of connexions between part of the pons and specific cerebellar lobes. Abbie (1934), on morphological grounds, felt that the 'post-trigeminal' part of the pons sends fibres to lobes anterior to the fissura prima, while the 'pre-trigeminal' is connected to the lobes posterior to this fissure. Masuda (1914) believed that the caudal part of the nucleus was connected to the anterior part of the cerebellum and the rostral part of the posterior cerebellar lobes.

It is interesting that when action potentials were recorded from the culmen following the stimulation of the pons the stimulating electrodes had been placed in the caudal part of the nucleus (Dow, 1939). Oscillographic studies following cerebral cortical stimulation tend to deny any highly specific relationship between topographical divisions of the cerebral cortex and cerebellum. Curtis (1940) states of his results: 'They indicate that there is no region in the neo-cerebellar cortex which can be said to be particularly related to any region of the cerebral cortex.' Curtis (1940) had found, and we have confirmed the results, that stimulation of a single point on the cerebral cortex may result in action potentials in widely distributed parts of the ponto-cerebellar projection area. In monkeys oscillographic studies suggest (Dow, 1942) that there is a predominance of the projection from so-called association areas to the lobulus ansiform as compared to vermian lobules. Further there is some evidence that the more rostral vermian lobules receive a richer projection from face areas and the more caudal lobules a richer projection from leg areas.

A tecto-cerebellar tract has been described by Hines (1925, 1929), Larsell (1936*a, b*) and others. It is said to enter through the medial part of the superior cerebellar peduncle. Its exact origin and termination are not known.

The three well-recognized afferent systems, (1) vestibulo-cerebellar, (2) spino-cerebellar, including trigeminal and reticulo-cerebellar connexions, (3) and the ponto-cerebellar connexions, although overlapping more or less extensively in certain areas (Fig. 9) tend to be restricted to certain parts of the cerebellum and thus to point toward a functional localization of the cerebellum on the basis of the afferent fibre connexions. This has been a constructive concept in cerebellar physiology (Bremer, 1935; Fulton & Dow, 1937) with important clinical implications (Bailey, 1933).

On the basis of most of the available data, none of these systems shows a definite topographical localization within its respective field except for the representation of the extremities predominantly on the homolateral side. Indeed, it appears that for all these systems a small part of each afferent system may be distributed throughout the extent of their respective projection areas.

Olivo-cerebellar connexions. The fourth afferent cerebellar system, the olivo-cerebellar connexions, seems to differ in many respects. It is connected not with a particular part of the cerebellum but with the whole of the cerebellar cortex, the fibres making up a large part of the restiform body. Furthermore, specific parts of the inferior olive and the dorsal and medial accessory olivary nuclei are connected with specific lobes of the cerebellum and apparently exclusively so. These facts were first indicated by the findings of Henschen (1907), Holmes & Stewart (1908), Zimmerman & Brody (1933) and others with pathological material, and recently in a most important review and experimental study in rabbits and cats by Brodal (1940*a*). The schema shown in Fig. 10, the data for which was taken from Brodal's paper, shows in detail the relationship between the inferior olive and the cerebellum.

Electrical stimulation in the immediate vicinity of the inferior olivary nuclei in the cat (Dow, 1939) results in the synaptic activation of the whole of the olive. This causes an action potential to appear throughout the whole of the cerebellar cortex. This response can be identified by an extremely well-marked conditioning effect which occurs in the olive. It was observed that from an identical lead point on the anterior lobe of the cerebellum, the stimulation of the olive produces a response which is greatly different in sign and shape from that produced by stimulating the reticular formation or the spino-cerebellar tracts. This suggested that the potential was occurring in different elements or in the same elements excited in a different manner. A relationship between this observation and the two types of afferent endings in the cerebellar cortex, namely, the mossy fibres and climbing fibres, immediately suggests itself. The fact that mossy fibre connexions are designed for a widespread activation of the cerebellar cortex makes one suspect that afferent systems showing no sharp topographical relationships may terminate as mossy fibres. The climbing fibres, on the other hand, connecting a single fibre to a single Purkinje cell, would be an ideal type of termination for an afferent system showing a point to point relationship between specific parts of its nuclei of origin and specific lobes

of the cerebellum. The olivo-cerebellar system, according to the work of Brodal (1940a), has such a point to point relationship.

Origin of mossy and climbing fibres. The endings of the afferent fibres in the cerebellar cortex are yet unknown. Reviews on the subjects are to be found in Ramón y Cajal (1909-11), Jakob (1928) and Kappers *et al.* (1936). Opinions based

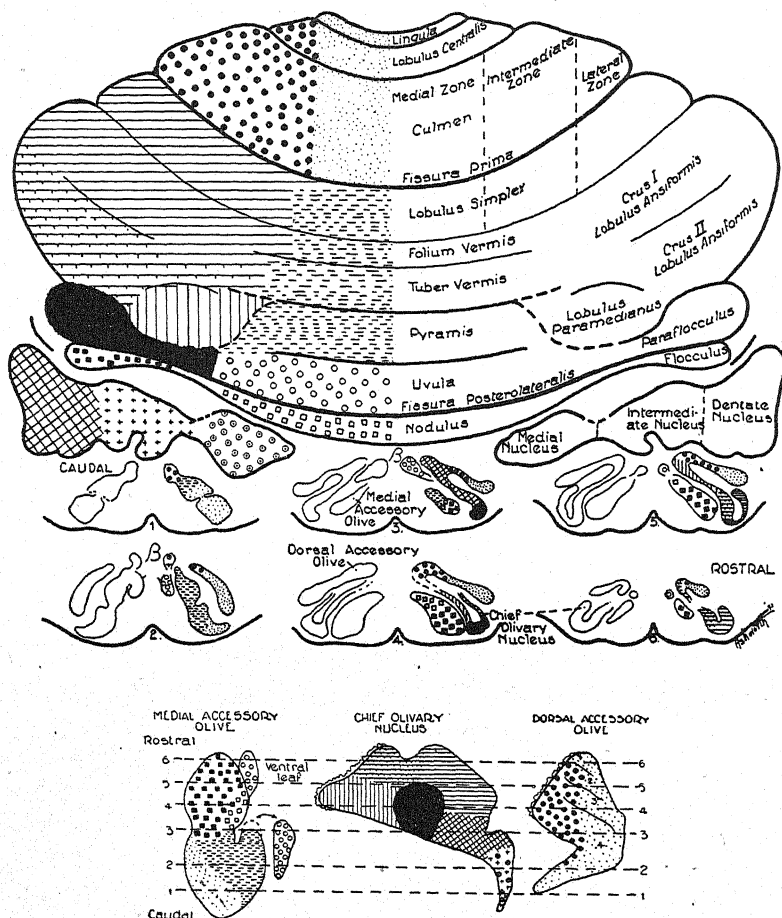


Fig. 10. Schema of the cerebellum to show the olivo-cerebellar connexions according to Brodal (1940a). The diagrams of the inferior olive are copied from Brodal's diagrams of the cat. The diagram does not show the connexions from the lateral zone of the anterior lobe to the chief olivary nucleus. This connexion is demonstrable in rabbits and monkeys in whom the lateral zone is well developed. This subdivision seems to be missing in the cat.

on Golgi preparations in young mammals, on pathological material and comparative anatomical data have been advanced by various workers, but none of these methods seems capable of solving the problem. Recent textbooks on neuro-anatomy, Ranson (1939), Larsell (1939), influenced by Ramón y Cajal, state that the pontine fibres entering through the middle peduncle probably end as climbing fibres and those entering by way of the inferior peduncle are thought to end as mossy fibres. Strong

(1929) suggests that an afferent system may end partly as mossy fibres and partly as climbing fibres. Lorente de Nó (1924) and Snider (1936) are of the opinion that the climbing fibres represent the endings of intracerebellar association fibres rather than afferent fibres from extracerebellar centres. Miskolczy (1931, 1934) and Snider (1936) have used experiment degeneration methods with success and their results will be discussed below.

The most logical view, in my opinion, is that vestibulo-cerebellar, spino-cerebellar and ponto-cerebellar fibres end as mossy fibres and olivo-cerebellar fibres end as climbing fibres. This is the only division which will account for the distribution of both mossy and climbing fibres throughout the whole cerebellar cortex if one is to assume that each represents endings of extracerebellar afferent systems and that each afferent system ends in a uniform manner. In support of this division are the observations of Snider (1936), who showed that section of the brachium pontis results in unequivocal degenerative changes in the terminations of mossy fibres. These changes have been confirmed by Snider in 1938 working in the Laboratory of the University of Chicago (Barnard, 1940). Similar changes in the mossy fibres have previously been described by Miskolczy (1931), following hemisection of the spinal cord.

Against such a suggestion are the observations of Miskolczy (1934) that, following midline medullary lesions which severed the olivo-cerebellar fibres, identical degenerative changes were found in the mossy fibres. These lesions could hardly have avoided cutting some of the reticulo-cerebellar fibres which, we have every reason to believe from oscillographic studies, end as do the spino-cerebellar fibres and could account for the changes described. The statement of Miskolczy (1934) that he found no evidence of degeneration of climbing fibres after his lesions of the medulla, if confirmed, is of course more significant.

One cannot deny the possibility favoured by Lorente de Nó (1924) and Snider (1936) that the climbing fibres are terminations of association fibres, possibly from the long collaterals of Purkinje cells. However, the short collaterals which can be followed in a single section end with ring-like endings about the basal parts of the dendrites and cell bodies of the Purkinje cells (Ramón y Cajal & Illera, 1907; Lorente de Nó, 1924). In silver preparations it is as impossible to follow these long collaterals to their termination as it is to follow fibres from the cerebellar peduncles into their termination in the respective folia.

(5) *Efferent fibre connexions*

Efferent connexions from the cerebellar cortex are provided by the axons of Purkinje cells. These fibres terminate in the subadjacent deep cerebellar nuclei, or in the case of the phylogenetic older lobes, directly in the vestibular nuclei and reticular formation of the medulla. The most recent study of the exact termination of these fibres from the various lobes of the cerebellum is that of Jansen & Brodal (1940). A complete review of the extensive literature on this subject may be found in their excellent paper. Fig. 11 represents diagrammatically the findings of these authors and those of Dow (1936, 1938a).

Jansen & Brodal (1940), following their experience with degeneration from small, well-localized cerebellar cortical lesions, emphasize the topographical relations between specific cerebellar lobes and specific subdivisions of the deep cerebellar nuclei. They liken this localization to that of the olivo-cerebellar afferent fibres. However, it should be noted that the Marchi method is not as satisfactory in determining the exact termination of fibres as is the method of retrograde cell changes employed in the olivo-cerebellar studies by Brodal (1940a).

A study of the diagrams in Jansen & Brodal (1940) shows a considerable overlapping in the terminations of the separate folia within the ten areas indicated. Certainly so far as the extracerebellar projection to Deiters's nucleus is concerned, there are terminations from widely separated parts of the cerebellar cortex.

The most lateral lobes, namely, the paraflocculus and lobulus ansiformis and the lateral zone of the anterior lobe and lobulus simplex are connected to the dentate nucleus. The intermediate zone of the anterior lobe and lobulus simplex, the medial part of crus I, lobulus ansiformis, and lobulus paramedianus, send fibres to the intermediate nucleus. The paraflocculus and the lateral part of the pyramis also send fibres to the intermediate nucleus, according to Dow (1936). The midline lobes (vermis) send fibres to adjacent parts of the medial nucleus (nucleus fastigii). The flocculus is the only part exclusively connected to extracerebellar nuclei, sending fibres to Deiters's and Bechterew's nuclei. In addition, there are some direct fibres from the nodulus, uvula and the anterior lobe to the vestibular nuclei and perhaps some from the pyramis as well (Jansen & Brodal, 1940). The more basilar parts of the vermian lobes seem to send a larger proportion of their fibres directly to the vestibular nuclei rather than to the fastigial (medial) nucleus. Some other important papers on this subject, which are discussed in detail by Jansen & Brodal (1940), are those by Clarke & Horsley (1905), Löwy (1916), Brouwer & Coenen (1921), Saito (1922, 1923) Hohman (1929) and Bender (1932).

(6) *Association fibres*

Although Clarke & Horsley (1905), Brouwer & Coenen (1921), Saito (1922, 1923) and Jansen (1933) using the Marchi technique have described long association fibres between remote lobes of the cerebellum, Dow (1936, 1938a) failed to find such fibres. Abundant association fibres to adjacent folia have been found by all workers. They may go for considerable distances when parts, originally contiguous, become separated by secondary development. Such are thought to be the fibres passing from the medial parts of the paraflocculus to the lateral part of the uvula and pyramis in the rat.

The association fibres from the lobulus ansiformis to the folium and tuber vermis described by Jansen (1933) in the rabbit may likewise represent in the adult a persistence of the local association fibres which remind one of their developmental relationships. These are not universally present from all parts of the lobulus ansiformis in the rat and cat as Dow (1936) was unable to find them following lesions in the lateral parts of these lobules. More medially placed lesions in the monkey, however, showed the presence of such association fibres (Dow, 1938a). As has been

emphasized previously, one must be sure that the lesion does not pass deep enough to involve incoming fibres.

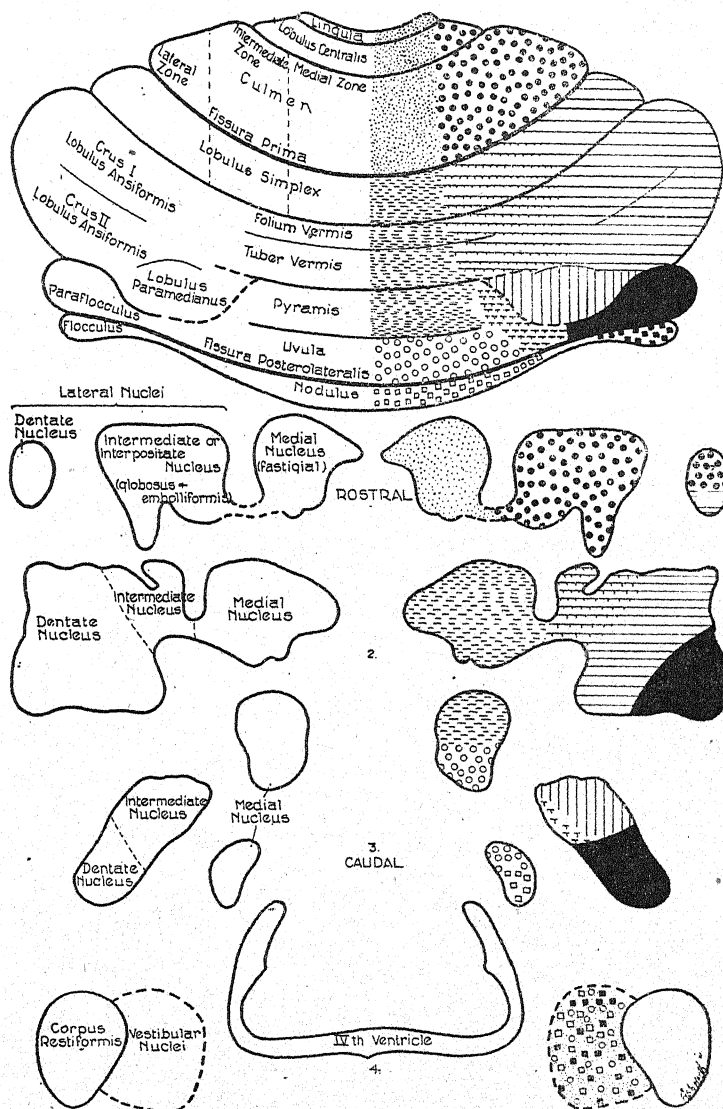


Fig. 11. Schema of the cerebellum to show cortico-nuclear connexions as given by Jansen & Brodal (1940) and Dow (1936, 1938a).

Besides the myelinated association fibres demonstrated by the Marchi technique, which may consist of collaterals and possibly axons of Purkinje cells, unmyelinated association fibres must be considered. Probably none extend for long distances, however. Dow (1938b) found that very strong stimulation of a cerebellar folium, sufficient to produce an after-discharge lasting several seconds, failed to modify the electrical activity farther than 5 mm. distance from the site of stimulation.

(7) *Cerebellar nuclei*

When one considers the deep cerebellar nuclei of all vertebrates, the nuclei may be divided into medial and lateral groups (Fig. 11). The former, according to most authors, is homologous with the fastigial nucleus of higher forms. Mussen (1927), Rasmussen (1933) and Abbie (1941), however, consider the medial group to consist of both fastigial and globose nuclei. The lateral group consist of intermediate or interpositate nuclei (globose and emboliform, according to most authors) and a most lateral part, the dentate nucleus. The term lateral nucleus has been used (Jansen & Brodal, 1940) synonymously with the dentate nucleus.

Development. The development of the deep cerebellar nuclei has been described in detail by Dowd (1929) in the pig. Previous work had consisted of a description of developmental stages in the human cerebellum by Weidenreich (1899), Vogt & Astwazaturow (1912), Brun (1917-18, 1925), Brunner (1919) and Demolé (1927). The first indication of the nuclei in pig embryos 50-60 mm. in length is an indistinct concentration of neuroblasts medial and lateral to the fibres from the vestibular nuclei which come dorsally into the cerebellum. The lateral nucleus is continuous ventro-medially with the cell masses of the superior vestibular nucleus. Although continued growth of these two nuclei occurs, no further subdivision occurs until between the 90-95 mm. foetus. At this stage there appears a more lateral expansion of the generalized lateral nucleus which appears to be the beginning of the dentate nucleus of higher forms. Adult relations are reached in pig foetuses of 200 m. length. At this stage a distinct ventro-lateral dentate nucleus is connected to the larger intermediate nucleus by a narrow bridge of cells. It in turn is joined by a less dense region of cells to the medial or fastigial nucleus. The cellular continuity between the ventro-medial parts of the intermediate nucleus and the superior vestibular nucleus is maintained.

As Dowd points out, this development recapitulates the order of phylogenetic development of the cerebellar nuclei and emphasizes their close relationship to the cells of the vestibular nuclei of the medulla oblongata. Larsell (1936*a*) has described briefly the development of the nuclei in the opossum where it differs in no essential from that of the pig, although maintaining an even more continuous relationship with the vestibular nuclei as was also pointed out by Voris & Hoerr (1932).

Divisions. A comparative study of the deep nuclei in various orders of mammals has been made by Weidenreich (1899), Van Hövell (1916), and Brunner (1919), Brunner (1919) stated there were two nuclei in Chiroptera and Insectivora, a medial and a lateral, while Larsell (1936*b*) described in *Myotis*, a small primitive bat, a beginning differentiation into the three nuclei of the higher mammals. All are agreed that at least three nuclei can be made out in marsupials, rodents, ungulates and carnivores.

Weidenreich (1899) believed the intermediate or interpositate nucleus could be subdivided into two divisions which he called the antero-lateralis and postero-lateralis in the sheep, dog, and cat. He considered them homologous to the nuclei emboliformis and globosus, respectively, of human anatomy. Snider (1940) found

a similar division in the rabbit and cat on the basis of cytological differences only. Dowd (1929) felt that there was some indication of a similar division in the pig, but did not consider it of great significance. Brunner (1919) denied the presence of such subdivisions below the primates. Allen (1924) and Jansen & Brodal (1940), on the basis of afferent and efferent connexions, found no grounds for division of the intermediate nucleus in guinea-pigs, rabbits and cats, although both considered the intermediate nucleus homologous with the emboliform and globose nuclei of higher forms. In the descriptions of both afferent and efferent connexions to the deep nuclei most of the discrepancies have been on the basis of differences in delimitation of the nuclei. This point is adequately discussed for the cerebellar cortico-nuclear connexions by Jansen & Brodal (1940). Mussen (1927), Rasmussen (1933) and Abbie (1941) subdivided the medial nucleus into two divisions, one of which they considered homologous to the globose nucleus. They consequently considered that the 'globose' nucleus sent its axons into the bulb via the uncinat fasciculus of Russell. Snider (1940) found a medial and lateral division in the fastigial nucleus in the rabbit and cat on cytological grounds. On the basis of their study of the cortico-nuclear relations, Jansen & Brodal (1940) 'find no reason for subdividing this part into two nuclei, a nucleus fastigii and a nucleus globosus as some authors do'. The final word on the homologies of the globose nucleus cannot be said until its fibre connexions are definitely determined in the anthropoid apes or in man. As is the case of lobes such as the tonsillâ, a study of closely graded developmental stages in the higher primates would be most illuminating.

There is embryological and histological evidence that the dentate nucleus of man consists of two parts, a dorso-medial older part, which is homologous to the so-called dentate nucleus of lower forms, and a very much expanded new part which comprises the bulk of the nucleus in man, the ventro-lateral part. These two parts differ in regard to cell types found (Gans, 1924; Demolé, 1927), in regard to iron reaction (Gans, 1924), embryologically (Vogt & Astwazaturow, 1912; Brun, 1917; Demolé, 1927), myelogenetically (van Valkenburg, 1912), and under pathological conditions (Brouwer, 1913*b*; Brun, 1917-18; Koster, 1926). There is not entire agreement as to whether the palaeodentate should be considered homologous to the nucleus interpositus of lower forms or as mentioned above with the dentate of lower mammals. This must again wait for accurate analysis of the fibre connexions and developmental stages of the separate cerebellar nuclei in the anthropoid apes and man. Certainly, as Jansen & Brodal (1940) point out, the extreme ventro-lateral part of the dentate nucleus expanded in those animals with a well-developed para-flocculus is not homologous with the ventro-lateral (neodentate) part of the nucleus in man.

Efferent connexions. Most studies of the efferent fibre connexions of specific deep cerebellar nuclei have been done on subprimate forms. Consequently the results must be described for the nuclei present in those species with the homologies for the human, still in doubt until the study is carried into higher primates. Marchi (1892), Probst (1901), Van Gehuchten (1905) and others described the course and distribution of the fibres of the brachium conjunctivum. Allen (1924), in the guinea-

pig, and Rasmussen (1933), in the cat, showed that cells in the intermediate (globose and emboliform, Allen), (emboliform, Rasmussen) and dentate nuclei send their axons out of the superior cerebellar peduncle (Fig. 12). These later authors also contributed to our knowledge of the termination of the brachium conjunctivum. A small descending portion of the superior cerebellar peduncle terminates in the

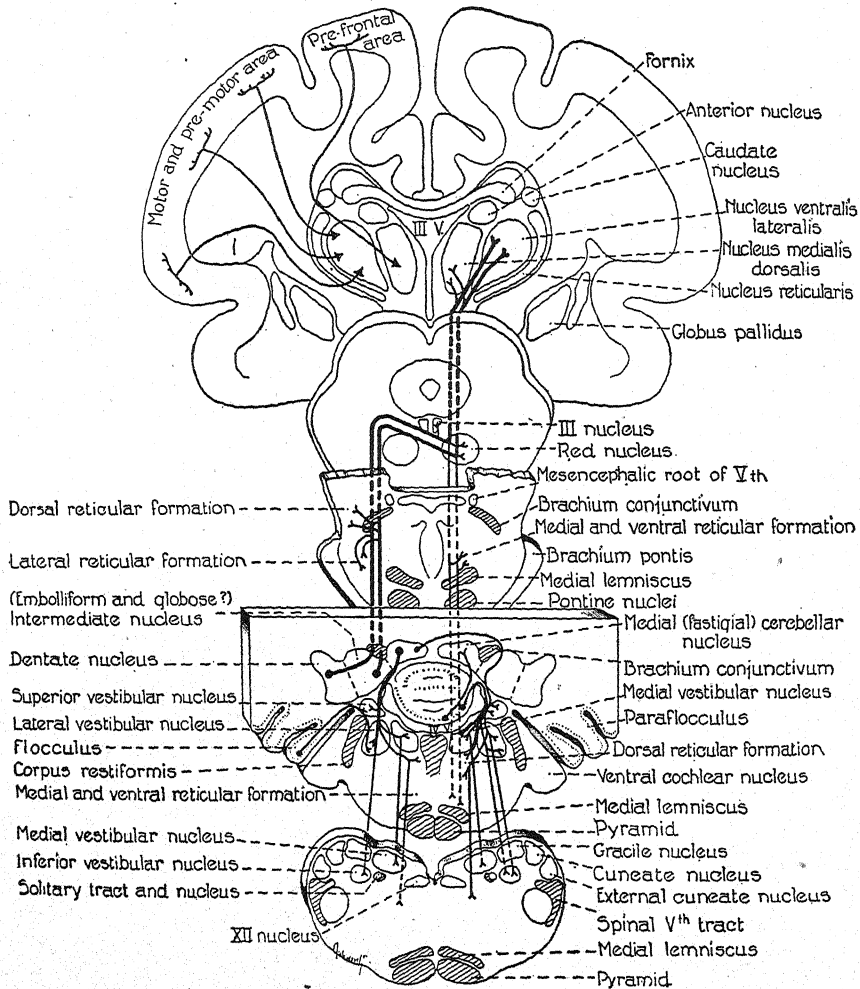


Fig. 12. Diagram of the fibre tracts leaving the cerebellum to terminate in extracerebellar nuclei. For description and discussion of controversial points see the text.

ventral and median formatio-reticularis of the pons and medulla. The main body of fibres form the ascending limb sending fibres to every part of the nucleus ruber and, a few to the region of the occulo-motor nuclei. Many fibres proceed farther cephalad into the thalamus where some, the brachium conjunctivum dorsale, terminate in the nucleus medialis thalami. The main band continues cephalad to terminate in the cephalic end of the largest and most lateral division of the nucleus

ventralis thalami. A few fibres are thought to be given off en route to the zona incerta, the median portion of the formatio reticularis ventralis, regio-subthalamis and to the grey matter dorsal to the most median division of the nucleus ventralis thalami (Allen, 1924). The difficulties of comparing the termination of these fibres in various species as described by the several workers are increased by the complications of nomenclature of the thalami nuclei. Walker (1938*a*) emphasizes that in primates the thalamic termination is restricted to the nucleus ventralis lateralis, though the fibres may reach the nucleus by several routes.

Clark (1936), Walker (1936, 1938*a*), and Crouch & Thompson (1938), in the *Macaca mulatta*, and Walker (1938*b*), in the chimpanzee, have recently studied the thalamic distribution of the degenerated fibres following lesions of the brachium conjunctivum. The findings in general agree with most of the earlier work on subprimate forms. It is of great interest that Clark (1936) and Walker (1936, 1938*a, b*) have pointed out that the nucleus ventralis lateral (Walker), in which most of the brachium conjunctivum fibres end in the primate thalamus, is the one which is connected to areas 4 and 6 of the cerebral cortex. Two recent pathological studies in human material confirming these cerebellar connexions to the thalamus and frontal lobe are those of Smyth (1941) and Freeman & Jaffe (1941). Although Mussen considered the thalamic termination of the brachium conjunctivum to be the same as the medial lemniscus, Allen (1924), Ranson & Ingram (1932), Clark (1936) and Walker (1936, 1938*a, b*) are all agreed that the two end in separate areas with practically no overlapping. Crouch & Thompson (1938) are less certain of this point from their preparations. Rasmussen (1933) failed to find degeneration in the ipsilateral midbrain following lesions in the intermediate and dentate nuclei, but considered this as an ascending portion of the uncinate fasciculus.

The medial nucleus (fastigial, Allen; fastigial and globose, Rasmussen) sends its fibres to the vestibular nuclei by way of direct fastigio-bulbar fibres and the crossed uncinate fasciculus of Russell (1895). Allen, working on the guinea-pig, states that the majority are direct and uncrossed, while Rasmussen, working in the cat, states that only one-tenth are direct and does not consider it justified to designate them separately from the uncinate fasciculus of Russell. They are agreed that the fibres terminate in the vestibular nuclei, the dorsal reticular formation of the medulla and by way of the median longitudinal bundle to eye-muscle nuclei. Mussen (1927) found a termination as above plus degenerations in other parts of the medulla which have not been confirmed by any other workers and probably represent pseudo-degeneration.

Although there is an important difference between the efferent connexions of the medial nucleus and the lateral group (interpositate and dentate) there is as yet no evidence that there are differences in the terminations of parts of these two complexes. One might assume on comparative evidence that the neodentate nucleus in the higher primates, including man, shows a progressively important dento-thalamo-cortical connexion, but no experimental evidence is available on this point. Here again isolated lesions in the various deep nuclei of the higher apes and man need to be studied. So far as the forms which have been studied are concerned, the

indications are that the degeneration from isolated lesions of dentate or interpositus results in degeneration terminating in the same areas of midbrain and thalamus. Allen (1924), following lesions restricted to each of these nuclei, states (p. 431): 'The axons from the nucleus intermedius (interpositus) and nucleus dentatus probably supply the same regions.'

Rasmussen (1933), following small lesions of these two nuclei, called by him the emboliform and dentate, says (p. 169): 'The most obvious result from these studies on the cat brain is the similarity in the degeneration picture which follows lesions in any part of the lateral group of nuclei.' He says further (p. 170): 'There is in this material no support for the contention of Marburg (1924) that nucleus emboliformis gives origin to fibres that terminate in nucleus magnocellularis of nucleus ruber, while those from nucleus dentatus end in parvocellularis (small cells of nucleus ruber) and the thalamus.'

In the light of the evidence available, the following statement of Jansen & Brodal (1940) seems hardly justified. They say (p. 315): 'The existence of a definite cortico-nuclear localization as determined in this investigation leads one to assume that a similar topographical correlation may exist between the cerebellar nuclei and the parts of the central nervous system to which the efferent fibres of these nuclei are distributed. This question, so far as we know, has up to the present not been investigated and calls for further research.'

To be sure, much more work needs to be done, particularly with new techniques, before one is justified in stating that topographical localization does not exist in the cerebellum. Indeed, the very challenging observation of Larsell & von Berthelsdorf (1941), which indicates that there is a very strong correlation between the percentage of total limb muscle weights of fore- and hindlimbs and the percentage of total area of the ansiform lobe in the crus I and II, respectively, in six different species of mammals, should provide further stimulus in efforts to find evidence of topographical localization.

V. SUMMARY

A review of previous work which has influenced cerebellar terminology is presented. An effort is made to harmonize the various sets of terms applied to lobes and fissures of the mammalian cerebellum with each other and with the terminology of human anatomy. The points in which agreement cannot yet be found and on which homologies with human anatomy are not clear are emphasized.

The phylogenetic development of the cerebellum is described with particular attention to the contributions of Larsell in Amphibia, reptiles and primitive mammals. A schema of the folial pattern which seems best suited for the whole vertebrate system is presented, which is designed to show fundamental morphological principles and at the same time to conserve those terms now in most frequent use.

The embryological development of the mammalian cerebellum is described in

of the postero-lateral fissure of Larsell and the fundamental difference between the flocculus and paraflocculus of the mammalian cerebellum is stressed.

A detailed description of the histology of the cerebellar cortex in mammals is given. Recent advances in the study of the afferent fibre connexions to the cerebellum are brought out and contributions of oscillographic studies emphasized.

Each afferent system is analysed in detail both as to origin and termination in the cerebellum. Particular attention is given to the important cortico-ponto-cerebellar system of the higher mammals and the most recent analysis of the olivo-cerebellar connexion by Brodal. The functional localization in the cerebellum on the basis of the afferent fibre connexions is pointed out. It is emphasized that the termination of the afferent fibres does not favour topographical localization within the respective vestibular, spinal and cortico-pontine parts of the cerebellum.

The common features of the vestibulo-cerebellar, spino-cerebellar and ponto-cerebellar systems are mentioned as well as the fact that the olivo-cerebellar system seems to be distinct from the other afferent systems. A suggestion is made that the mossy fibre endings are from the vestibular, spinal and pontine systems, while the olivo-cerebellar system terminates as climbing fibres. Available evidence, both for and against this conception, is discussed in some detail.

Recent findings on the cerebellar cortico-nuclear connexions are presented. The development of the mammalian cerebellar nuclei and their homologies with the nuclei of the human cerebellum are discussed. It is pointed out that until such homologies are better established than at present it is impossible to transfer data from animal experiments to man in so far as the connexions of the globose nucleus is concerned. The efferent connexions of the deep cerebellar nuclei, as determined experimentally, are described in detail. The lack of available anatomical evidence for topographical localization on the basis of efferent cerebellar connexions is pointed out.

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VII. ADDENDA

The following remarks should be added to the top paragraph on p. 202: Blakeslee, Freiman and Barrera (1938) found that cells of the nucleus lying between the descending trigeminal root and the inferior olivary nucleus, called by them the nucleus lateralis medullae, send their connections to the cerebellum via the homolateral restiform body in the monkey. Blakeslee and Freiman (1940) have given a preliminary report indicating by the method of retrograde cell changes following restricted cerebellar ablations in the monkey that this cerebellar projection is to the homolateral half of the medial part of the "anterior vermis" and to a lesser extent to the "posterior vermis". A questionable projection of the "flocculus" was also suggested.

The reader's attention is directed to another important paper which came to my attention too late for critical evaluation in the body of the article. Stefanelli (1939) has reported comparative morphological observations in cyclostomes, fishes, and amphibians. An excellent bibliography of the literature in these lower forms is also included.

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PRODUCTION OF LIFE IN THE SEA

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I. INTRODUCTION

AN attempt is here made to collate the results of recent investigations which bear, directly or indirectly, upon the quantity of organic matter produced by plants in the open sea. The productivity of any extensive water mass has been defined as the quantity of organic matter produced by the phytoplankton over a period of time, such as a year. This organic matter provides animals and bacteria with food; its quantity is dependent upon the number of times during the year that plant organisms are produced, consumed, and their nitrogen and phosphorus set free in a form and at a depth where they can again be used by the phytoplankton to synthesize organic matter. It has long been known that their growth may be limited by shortage of available nitrogen compounds or of phosphates.

The standing crop of phytoplankton, or breeding stock, at any time is merely a 'momentary balance' between the processes of production and of consumption by animals and bacteria. The concentration of nutrient salts in the water mass at any time is likewise a momentary balance between their regeneration by animals and bacteria and their consumption by the phytoplankton.

The quantity of organic matter produced during any extended period of time is dependent upon a series of conditions many of which are themselves closely interrelated. In comparing two different water masses, a smaller average standing crop of plants and lower concentration of nutrient salts does not *necessarily* mean a smaller production of organic matter if other conditions allow more rapid growth of the plants. Such conditions are light, temperature, growth which extends to

greater depths, and rapid regeneration of their contained nitrogen and phosphorus within the photosynthetic zone in forms which can be again utilized, or alternatively brought by turbulence into the zone from below.

Any study concerning productivity necessitates the comparison of one area with another and involves comparison of quantities. The first is complicated by the constant movement of the water occupying an area, its slow replacement by a different body of water, and often the more rapid replacement of the upper layers. The comparison of quantities involves sampling over wide areas, usually to considerable depths, of animals and plants frequently distributed in swarms or patches. To complicate matters still further there is a production of minute plant organisms, no more than a few microns in size, in some areas at some periods of the year.

The assessment of the annual production of plant organisms does not lend itself to direct attack, but a consideration of the factors involved is beginning to shed light upon its magnitude.

It is generally assumed that in water masses where the annual production is great, the density of the animal population will be great; this assumption is roughly borne out by general observation. A series of quantitative observations are now indicating a relation between the fluctuations in production from year to year and the animal population.

With these considerations in view it is pertinent to list and discuss factors which affect, or may affect, the growth rate of phytoplankton in the sea, and at times slow down growth and so limit production. The list is probably still incomplete. Most of the factors are interrelated and some have an optimum value beyond which production is hindered. It is the mosaic of such factors, constantly changing, which controls production in nature.

II. PHYSICAL FACTORS AFFECTING THE GROWTH OF PHYTOPLANKTON

In nature the light falling on the sea is continuously changing with time, and as it penetrates the water it changes rapidly in spectral composition. In the clear blue water of the open ocean containing the least suspended matter and organisms, the blue and blue-green penetrate deepest. As shores are approached and more suspended particles are encountered in the water there is, in addition, a progressively greater loss due to scattering of light by these particles, particularly of light at the blue end of the spectrum. In a turbid estuary the loss from this cause becomes so great that blue light penetrates less deep than that from the red end of the spectrum. The change in composition is reversed (Cooper & Milne, 1938, 1939). Differences in transparency of the sea have a great effect both on the depth to which light penetrates and on the composition of the light at successive depths. It is noteworthy that in the transparent water of the Mediterranean sessile algae obtain sufficient light to live at depths exceeding 100 m.; experiment with a diatom culture in the Sargasso Sea indicated considerable photosynthesis taking place below 100 m. during daylight (Clarke, 1936). In the less transparent waters of temperate seas the photosynthetic zone is very much shallower.

With daylight the intensity of any one group of wave-lengths is roughly proportional to the intensity of all the groups in the whole spectrum. Hence the intensity of the whole spectrum can be measured by a photoelectric cell sensitive to a particular group of wave-lengths which has been calibrated against a standard light source. The value of the intensity (usually expressed in metre-candles or lux) is a function not only of the intensities of the groups of wave-lengths which make up the spectrum of daylight but also of the group of wave-lengths to which the particular photoelectric cell is sensitive and the occurrence of this group in the particular light source against which it has been calibrated. Photometer readings therefore do not give a directly proportional comparison between daylight and artificial light or daylight which has penetrated to a depth in the sea and changed in spectral composition.

These considerations delineate the value of experiments on the growth of phytoplankton in artificial light and indicate some of the complications involved in interpreting observations made in the sea.

With regard to the utilization by phytoplankton of light of different wave-lengths there is a dearth of information. Stanbury (1931) grew a marine diatom in daylight which had passed various coloured light filters and found that growth was not proportional to the percentages of the total incident daylight which passed through the filters (the intensity of the light reaching the diatoms) but was more nearly proportional to the amount of light energy which reached them. The energy is a function of intensity and spectral composition of the light.

A number of investigations have been made concerning the range of light intensity necessary for the growth of phytoplankton and concerning their growth rate at varying depths in the sea, both for short periods and over a period of 24 hr., during days in summer and winter.

Gaarder & Gran (1927) kept a mixed community of diatoms, freed as far as possible from animals, in flasks hung at different depths in Oslo Fiord in March. Below about 10 m.—the compensation point—their respiration exceeded assimilation. Marshall & Orr (1928) made similar experiments with a culture of the diatom *Coscinosira polychorda* in Loch Striven, finding that the compensation point over 24 hr. lay between 20 and 30 m. deep in summer and near the surface in winter. Close to the surface growth was inhibited during the hours of bright light in summer. Similar results were also obtained with a summer species of *Chaetoceros*. They noted that optimum growth took place throughout a range of a few metres, below which it decreased until the compensation point was reached.

Similar experiments were made by Jenkin (1937) in the English Channel using a culture of the diatom *Coscinodiscus excentricus*. Over 24 hr. in the summer greatest growth took place between $2\frac{1}{2}$ and 10 m. depth, the compensation point in this more transparent water lying at about 45 m. Photometer measurements at different depths were made during the course of these experiments and from them the flux of light energy was calculated. The oxygen production by the diatoms showed a linear relation to the flow of light energy through the water when this was between 7.5 joules or 1.8 g.cal./cm.²/hr. The compensation point lay at 0.55 joule.

Above 7.5 joules the linear relation ceased. In the shorter-period experiments maximum oxygen production occurred in a range between about 12 and 30 joules/cm.²/hr., and at greater intensities inhibition became apparent, actual contraction of the chloroplasts probably taking place at an energy flux of some 40 joules/cm.²/min. In the region of the optimum there were marked differences between the various experiments. Additional experiments with *Biddulphia regia* gave similar results. Other investigators have determined the light intensity at the compensation point, obtaining photometer measurements of 100–500 lux, in rough agreement with the value of 0.55 joule/cm.²/hr. which is equivalent to about 360 lux of daylight.

It is noticeable when growing diatoms in culture that their behaviour and growth rate depend to a large extent upon their previous history, whether they have been making rapid or slow growth before being introduced into the culture medium, that is to say upon their 'physiological state'. The following experiment (Harvey, 1939) shows both this and the effect of temperature on growth rate. A culture of *Biddulphia mobiliensis* was divided into two parts. One was then grown in a north window in relatively dim December light. The other portion was grown near an electric bulb immersed in a bowl of running water; it received continuous light of some 18,000 lux measured with a photometer. At the end of a week subcultures of each of these cultures were made in sea water enriched to the same extent. Each subculture was divided into ten glass vessels. They were immersed in two water-baths, one kept at 13° C., the other at 18° C., at different distances from an electric bulb which was immersed in each bath. The light which each vessel of culture received was measured. After 72 hr. continuous light the percentage increase in number of cells which had taken place during the 72 hr. was determined.

	Temp. ° C.	Percentage increase in lux in 72 hr. at				
		28,000	18,000	8000	4100	1400
Cells grown previously in dim light	18	7	68	66	106	87
	13	24	16	14	8	2
Cells grown previously in continuous light at 18,000 lux	18	171	236	190	123	98
	13	31	70	105	67	36

Barker (1935) has investigated the effect of temperature on two species of the diatom *Nitzschia* and several dinoflagellates. The optimum varied for the different species; below the optimum there was a roughly linear relation between temperature and growth rate.

These various observations indicate that phytoplankton has sufficient light to make active growth down to a depth of more than 100 m. in the clear blue transparent waters of the open sea in summer or tropic light; as the shores are approached this photosynthetic zone rapidly shallows, and in estuaries may be no more than a few metres deep. During the short days of midwinter in temperate regions the quantity of light energy entering the sea daily is only a fraction of that entering daily in the summer, about one-ninth in the English Channel area (Atkins, 1939). It does not follow that the quantity of light available for photosynthesis in the sea

is nine times less in winter than in summer. At this position about one-half of the light entering the surface penetrates to a depth of 5 m., and one-ninth to a depth of some 16 m. Thus the photosynthetic zone averaging 45 m. deep for the 24 hr. in summer would shallow and average some 16 m. in winter—still sufficient for considerable growth to take place. Considerable growths during winter in temperate seas are unusual but they have been observed in relatively shallow waters. Thus Bigelow (1926, p. 396) and Fish (1925) have reported heavy growths of diatoms during December in Cape Cod Bay and at Woods Hole.

In nature there is yet another physical factor besides light and temperature which obviously affects production. Vertical mixing of the water is continuously taking place through turbulence or eddy motion. This has a dual effect, increasing or hindering the growth of plants. Without it the photosynthetic zone would soon be depleted of nutrient salts except in shallow areas and life would cease through lack of supplies from below. Incidentally the whole physical state of the oceans and the world's climate would change, the viscosity of the water becoming laminar.

Such eddy motion or turbulence is set up by wave motion, by cooling and evaporation at the surface causing convection currents, and also by a current meeting even a low submarine ridge or bank. The freedom of the eddy motion to penetrate from the surface downwards is reduced where the density of the water increases with depth, that is, with increasing 'stability' of the water column. Unfortunately, the amount or degree of turbulence (coefficient of eddy conductivity) rarely lends itself to direct measurement in the sea.

There are some areas where the effect of turbulence bringing nutrient salts into the photosynthetic zone is reinforced by water upwelling from below to take the place of surface layers which are being drawn away in a current. This is particularly well marked in two areas off the tropical west coast of Africa, where the plankton is singularly abundant (Hentschel & Wattenberg, 1930) and off the west coast of South America (Gunter, 1936).

With regard to turbulence hindering production, plant organisms are continuously being carried down from the levels where they are making most rapid growth, and if carried below the photosynthetic zone are then lost to the breeding stock and furthermore lose substance through respiration.

The losses from respiration will presumably deplete the food reserves, which are oils, and may increase the specific gravity of the plants if they have remained for a period below the zone.

This continuous drain on the rapidly breeding plants is likely to be most where the turbulence is greatest and the photosynthetic zone shallow, with a great depth of water below it.

Marked diminution in production has been attributed to this factor in the Bay of Fundy by Gran & Braarud (1935) and in deep Antarctic waters by Hart (1934). In these areas there is always an ample supply of nutrient salts in the upper layers.

In many shallow areas turbulence also acts indirectly in hindering plant growth by rendering the water turbid with detritus brought up from the bottom and kept in suspension. This is marked in the Bay of Fundy in the immediate vicinity of

South Georgia (Hart, 1934) and indeed in most shallow areas where there are strong tidal currents and a bottom of finely divided particles.

It seems probable that turbulence plays a large part in limiting production, particularly during the winter in all temperate seas.

Thus a condition for greatest production occurs in the sea where the relation of turbulence to the quantity of incident light is at an optimum.

III. CONCENTRATION OF NUTRIENT SALTS

In nature the growth of phytoplankton frequently reduces the concentration of phosphate to below 1 mg./m.³, and the concentration of available nitrogen compounds—nitrate, nitrite, ammonium, uric acid, urea and probably amino-acids—to a small value. Hence it is pertinent to consider how the approach to low concentrations affects the rate of growth.

Experiments with the diatom *Nitzschia closterium* have shown a marked reduction in rate of photosynthesis with phosphate concentrations below about 10 mg. P/m.³, most marked below 5 mg. P/m.³ (Harvey, 1933). Ketchum (1939*a*), using the same species, has concluded that the rate of growth is independent of phosphate concentrations above 17 mg. P/m.³, below which it falls off. In water with ample phosphate the rate of division was independent of nitrate concentrations above 47 mg. N/m.³, the lowest concentration examined.

The same author (1939*b*) has found that when this diatom is grown in a medium lacking phosphate, phosphorus-deficient cells are formed. These absorbed phosphate in the dark when phosphate was added. The alga *Chlorella pyrenoidosa* when grown in a medium lacking nutrient salts formed cells containing only a third of the normal phosphorus and half the normal nitrogen. This suggests that in nature utilization of nutrient salts from water having very low concentrations may proceed continuously, while carbon assimilation takes place only during daylight. Such a mechanism allows growth at greater dilutions of nutrients than would otherwise be possible, and the most efficient use of short periods of illumination in waters depleted of nutrient salts.

The growth rate of plants is reduced by low concentrations of nutrient salts in solution in the water in many areas of the sea, but there is no reason to suppose it is ever brought to a standstill since both phosphate and available nitrogen compounds are continuously being reformed. Analyses have shown the phosphate concentration to be sometimes reduced to less than 0.5 mg. P/m.³, with a small concentration of nitrate + nitrite; at other times and places the latter have been found at less than 1 mg. N/m.³ with a small concentration of phosphate. This latter condition does not necessarily mean that the final limit has been brought about by lack of available nitrogen because it is possible and indeed likely that the water contained ammonium and amino-acids. Experiments with plankton diatoms grown in water to which both nitrate and ammonium had been added showed that they utilized ammonium in preference to nitrate (Harvey, 1940). Indeed, it appears doubtful whether the change of ammonium to nitrite and nitrate, which takes place

in nature, plays any part in influencing the productivity of the sea or the kind of plants and animals in it.

The concentration of nutrient salts change together in the sea, the ratio of phosphate to nitrate + nitrite remaining within fairly narrow limits over very wide areas (Redfield, 1934; Cooper, 1937). For this reason the concentration of phosphate usually affords a close index of the concentration of both nutrients in the water. There are, however, some water masses where the ratio of N to P differs considerably from the customary value of 9 to 1 by weight, which is also the ratio in which the two elements tend to be present in phytoplankton organisms.

In temperate and arctic seas plant life is at a minimum during the winter months, and incidentally the zooplankton is also at a minimum, there being a great diminution during the autumn. Towards the end of the winter a maximum of phosphate and nitrogen salts, by that time mostly nitrate, become accumulated in the water and tolerably well distributed from top to bottom owing to vertical mixing brought about by cooling at the surface and consequent convection currents. The magnitude of this 'winter phosphate maximum' is of particular interest since it has been found to fluctuate from year to year in the same area.

Phosphorus occurs in the sea in three forms: as phosphate in solution, as organic compounds in solution and in plants, animals and detritus. At the time of the winter maximum the investigations of Redfield *et al.* (1937) indicate that the quantities present in organic compounds in solution and in living animals and detritus are both much reduced.

Percentage of total phosphorus in upper 60 m. of the Gulf of Maine

	Aug. 20	Feb. 26
Phosphate	61.2	89.5
Organic phosphorus compounds in solution	30.1	6.4
Organisms and detritus	8.7	4.1
	100	100

Production in the sea is slowed when the *concentration* of nutrient salts falls to low values, but it is the *rate of replenishment* of the nutrients in the water of the photosynthetic zone which ultimately limits the production. The quantity of nutrients in the photosynthetic zone—a momentary balance between utilization and replenishment—is a measure of the potential production in the immediate future. There are good grounds for supposing that this quantity is used and used again, perhaps several times during the course of a year, in forming plant tissue. Hence it is the rate of replenishment which ultimately controls production.

IV. SUPPLY OF IRON, SILICATES AND CARBON DIOXIDE

There are grounds for expecting that the growth of phytoplankton may be limited in some parts of the open oceans by an insufficient supply of iron. Only a few milligrams per cubic metre can be found by analysis in sea water. Seiwel (1935) could detect none in the upper 40 m. layer at a position in the Atlantic by

a method which would detect 1–2 mg./m³. Cooper (1937) concluded that less than 10⁻⁷ mg. of iron ions per m.³ could exist in sea water in equilibrium with ferric hydroxide, owing to the insolubility of the latter. It is probable that most of the small amount of iron in solution exists as colloidal micelles of ferric hydroxide and possibly ferric phosphate.

Phytoplankton organisms yield on analysis relatively large amounts of iron, several times more than their phosphorus content (Cooper, 1935), very much more than they could obtain from iron ions in solution. There is evidence that most of this is in the form of ferric hydroxide; and possibly ferric phosphate, adsorbed on the surface of the organisms.

Experiment with diatoms in culture has shown that they are able to obtain their requirement for growth when this is added in the form of particulate ferric hydroxide, from which it may be concluded that they possess some mechanism for dealing with the extremely insoluble hydroxide at the cell-water surface. The actual quantity of iron required *within* the cell is probably very small; experiment with cultures showed that the addition of 1 mg. of iron to an iron-deficient medium led to a growth similar to that brought about by the addition of 175 mg. phosphate-phosphorus to a phosphorus-deficient medium, provided that the addition was made as recently formed colloidal hydroxide (Harvey, 1937). The inference is drawn from these observations that a fraction of a milligram of iron per m.³ in the sea should suffice for phytoplankton growth, provided this is present as small colloidal micelles of the hydroxide such as are formed when many organic compounds of iron hydrolyse at great dilution in sea water. The rate at which these aggregate to form larger micelles and the nature of the iron voided into the water when phytoplankton is eaten by animals must both play a part, for it is the particle size of the iron hydroxide in the water, as much as its concentration, which is likely to determine whether a water is deficient in this element.

The colloidal ferric hydroxide, formed in sea water when iron salts hydrolyse at great dilutions in the presence of polyhydroxy organic compounds, obtains a considerable measure of protection from aggregation due to the influence of the organic compound. Of many iron salts of polyhydroxy anions examined, ferric ascorbate, the salt of vitamin C, hydrolysed to the least unstable colloidal hydroxide. Such 'protection' of the colloidal hydroxide doubtless plays an important part in reducing aggregation and flocculation in nature.

The total iron found by analyses of various sea waters, considered in relation to the high ratio of iron to phosphorus in analyses of diatoms, indicates that all the iron in offshore water is collected by phytoplankton organisms several times during the year. Colloidal ferric hydroxide is readily adsorbed on many organic surfaces, not only phytoplankton organisms. It would seem likely that the sequence of events which maintains the presence of colloidal ferric hydroxide in the upper layers of the oceans beyond the influence of land drainage follows a course such as adsorption of the hydroxide on the surface of plankton organisms which, on being eaten and digested, is redissolved and voided into the sea as a ferric salt together with soluble or colloidal organic matter; as this mixes with the alkaline sea water ferric hydroxide

is formed from these iron salts, and this is 'protected' from aggregation and flocculation by the organic matter voided with the salts. Unless there is some such cycle of events it is difficult to understand why the upper layers would not become depleted of iron within a few years or less.

The silicate present in sea water can be estimated down to concentrations of about 10–15 mg. SiO_2/m^3 . The utilization of silica by diatoms occasionally reduces the silicate content of the upper layers of the sea to such a value. There is no evidence at what concentration the growth rate of diatoms is slowed through a short supply of silica. In the English Channel some 4 miles offshore, Cooper (1933) finds the silica content of the water reduced from a winter value of 300 mg. SiO_2/m^3 to 100 mg. by the end of April, after which it rises. At this position considerable and rapid growths of diatoms occur during May and June; later, as in most north temperate latitudes, the diatom community gives way to a flora in which peridinians with chitinous skeletons predominate. The rapid increase in silicate which occurs during the summer shows that it is quickly regenerated by solution of diatom frustules.

Hart (1934, 1941) and Clowes (1938) consider that short supply of silica slows the rate of diatom growth in the Antarctic, where concentrations fall from some 2000 mg. SiO_2/m^3 to very low values in some areas during the summer. Thin-walled diatoms occur at this time, and there is a correlation between the silicate content of the water and the silicification of the diatom *Corethron*.

There is an abundant supply of carbon dioxide in the sea, mostly in the form of bicarbonate. In the surface layers it exerts a partial pressure of the gas similar to its partial pressure in the atmosphere (Buch, 1939). However, an outburst of phytoplankton sometimes occurs utilizing 1.5–3 c.c. of carbon dioxide per litre of water, raising the pH of the water by *circa* 0.15–0.2. Such a change may lower the partial pressure of the gas by as much as 50%. In view of this large effect it is pertinent to consider the relation between the supply or partial pressure and the growth rate of phytoplankton.

Barker (1935) investigated the rate of photosynthesis of a fresh-water diatom and of a unicellular alga in relation to the partial pressure of carbon dioxide, finding little difference unless the pressure fell below about one-quarter of its value in the atmosphere. Experiments with the marine diatom *Nitzschia closterium* in waters of different hydrogen-ion concentration indicate that there is an ample partial pressure of carbon dioxide for photosynthesis to proceed at or near the maximum rate in waters likely to be met with in the open sea (Harvey, 1933; Barker, 1935). This species and some others continue to live and multiply in cultures which attain a singularly high pH, whereas the majority of planktonic species die out under such extreme conditions (Harvey, 1940). Nevertheless, these planktonic species live and multiply in culture using carbon dioxide when its partial pressure can be only a small fraction of the value likely to occur in the open sea. These various observations provide no indication that growth in the sea is materially affected by the carbon dioxide supply.

V. OTHER CHEMICAL FACTORS

Experiments have been made with the diatom *Ditylum brightwellii*,² growing it in water collected from the open sea which had been heated to 90° C. and enriched with phosphate, nitrate and iron. In some samples of water treated in this manner consistently good growth of the diatoms was obtained. In some samples, the diatoms with which the waters were inseminated formed auxospores and ceased growth unless a small quantity of *manganese* was added, from 1 to 2 mg./m.³ being sufficient. In other samples the growth rate of this species and of *Thalassiosira gravis* was materially increased by adding manganese, provided that the intensity of illumination was low or reduced to a few hours daily. Analyses of water from the Pacific by Thompson & Wilson (1935) show it to contain a variable small quantity of manganese, ranging between 1 and 10 mg./m.³, so it is possible that lack of manganese may play some part in nature.

No growth, or only very slight growth, of *Ditylum* was obtained in a series of samples of water collected during the summer months unless some compound containing divalent sulphur was added. Of such cystine, glutathione, methionine, thiamin and sodium sulphide rendered these waters fertile for this species, or possibly strain, of diatom (Harvey, 1939).

Sea water contains much sulphate, and experiments with a number of planktonic diatoms failed to show that this did not suffice for their growth, although the addition of cystine or sodium sulphide had the effect of increasing the growth rate of several species. In this connexion it is of interest that other organisms prefer to take in their sulphur requirements as divalent sulphur; Mast & Pace (1935) found that a colourless flagellate grew more rapidly in media where the sulphur was supplied as sodium sulphide or cystine than in media where it was in the form of sulphate.

Evidence has been obtained (H. Rogers, private communication) of organic matter containing divalent sulphur in solution in inshore sea water.

There is a possibility that other trace elements, besides iron and manganese, may at times occur in insufficient quantity for maximum growth of phytoplankton. Some evidence suggesting this has been obtained by myself. Of several trace elements necessary or beneficial to higher plants, such as boron, zinc and copper, there is an ample supply in sea water, but of several others known to have some effect the quantities found by analyses (Wattenberg, 1938) are below 1 mg./m.³, for instance, molybdenum.

The need for some organic constituent in the water was indicated in Allen's (1914) classic experiments on diatom growth in artificial sea water and I have obtained further evidence to the same effect. Matsudaira (1939) found that the growth rate of two species of diatom varied markedly in waters collected from several positions and depths, which had been enriched with nitrate, phosphate, silicate and iron. He concludes that the differences are due to some organic substance.

VI. CONSUMPTION OF PHYTOPLANKTON BY ANIMALS

The standing crop or breeding stock of phytoplankton is always in the process of being depleted by herbivores, but the incidence and intensity of this grazing is irregular. The abundance of herbivores, for the most part copepods, changes throughout the year; brood of any one species follows brood at intervals of weeks or months. Moreover, they occur in swarms. In their early stages these herbivores are small in comparison with most of the diatoms which compose the bulk of the phytoplankton in temperate regions, and the kind of food they subsist on then is unknown. In some localities there are many minute flagellates and minute diatoms in the water, but this is not so everywhere. Their survival and growth to a size when they can eat average-sized phytoplankton, such as is caught in a tow-net in most seas, will depend largely on the supply of food during these early stages.

Where and when herbivores of a diatom-eating size are numerous, there we would expect diatoms to be sparse and vice versa. This inverse relationship has been found to occur in several areas (Harvey *et al.* 1935; Wimpenny, 1936; Bigelow *et al.* 1940; Mare, 1940; Hart, 1941). On the other hand, Steemann Nielsen (1937) has found areas where large quantities of both zooplankton and phytoplankton exist simultaneously throughout a considerable period.

Hardy & Gunther (1935) have advanced a theory that zooplankton avoid areas rich in diatoms, and suggest that the animals do not rise at night into the upper layers, as they normally do, until currents carry the diatom-rich patch in the water above away from them. As a result of this 'animal exclusion' the animals and plants of the plankton would become distributed in alternate communities. However it may come about, such a distribution in alternate communities appears to be more usual than otherwise.

The density of phytoplankton often increases very rapidly, a spectacular rise in numbers taking place in a few days, particularly during the spring outburst in temperate seas. On the other hand, the rise in numbers of copepods of diatom-eating size depends upon conditions which existed while they were passing through their earlier stages some weeks previously and upon the proportion of carnivores which normally prey upon them. Thus the actual number of these later stage copepods present at any time depends upon past conditions, a factor which has been particularly stressed by Steemann Nielsen (1937) and by Clarke (1939). The effect of grazing herbivores on the plant community is brought about in a few days, whereas the effect of phytoplankton production on the abundance of herbivores depends upon a food supply during previous weeks or months; they are two different and quite distinct considerations.

Reverting to the effect of grazing, it was observed that the sudden diminution in numbers of diatoms at the end of the spring outbursts of 1933 and 1934 in the English Channel occurred at a time when the larger copepods had commenced to increase rapidly. At these times the water contained many faecal pellets of copepods coloured green with chlorophyll. These larger copepods are extremely voracious.

I have watched a late stage *Calanus finmarchicus*, kept moving in a suspension of diatoms, which discharged green faecal pellets at 20 min. intervals.

The observations made during these two years indicated that the sudden cessation of the vernal diatom outburst, which took place before half the available nutrient salts had been utilized, was due to their being eaten. Quantitative observations from other areas, of both phytoplankton and zooplankton, bearing upon the cessation of the vernal outburst have not been published.

The sudden cessation of the spring outburst, usually of several species of diatoms, is common in temperate regions and takes place well before the available nutrient salts are used up. That it is not due to lack of some other necessary chemical constituent in the water, with the possible exception of iron, is indicated by the following observation. If sea water collected during spring, or early summer, is enriched with iron and some twenty times more nitrogen salt and phosphate than it ever contains normally during the winter maximum and kept in the light, the diatoms present increase. If washed and filtered air is blown through the water, the growth of diatoms uses up all these added nutrients. The composition of the crop is similar to the composition of phytoplankton in the sea; the dominant species remain dominant and those which were less plentiful do not appear to be crowded out (Harvey, unpublished). It yet remains to be determined whether water from the open sea contains sufficient iron to produce such heavy growths.

Some experiments have been made on the rate at which late-stage *Calanus*, a particularly frequent herbivore, grazes on suspensions of diatoms of varying concentrations, mostly more concentrated than would occur in the sea. This animal collected in 1 hr. all the *Nitzschia*, a very small diatom, present in 0.2 c.c. of the water, irrespective of the concentration of the diatoms. With two species of larger diatoms it collected those in *circa* 3 and 8 c.c. respectively (Fuller, 1937; Harvey, 1937). With reference to the products of digestion Gardiner (1937) has observed the rapid liberation of phosphate by *Calanus* feeding on diatoms, and Cooper has found that the faecal pellets contain very little phosphorus compared with uneaten diatoms. The nitrogen is probably excreted mostly as ammonia, but how much is digested and how much left in the pellets is unknown.

In the older literature there appears a tacit assumption that the majority of phytoplankton organisms die and sink, and that in temperate seas there is a continuous rain of dead diatoms falling on the bottom. That this does occur in some areas is known, but in one such area Moore (1931) has shown that the quantity of diatoms reaching the bottom is greatly exceeded by the quantity of green faecal pellets. I am indebted to Dr T. J. Hart for the information that the diatomaceous ooze lying on the sea floor of considerable areas of the South Atlantic below very deep water consists of much broken frustules, and is in appearance compatible with their having been eaten and voided by euphausiids in the upper layers. It seems indeed likely that the fate of most phytoplankton organisms is to be eaten before reaching the bottom, particularly in deep water.

The digestion of the plant cells and the subsequent history of the solid excreta is a link in the food chain which merits investigation. Doubtless particles of

food, not been fully digested by the herbivores which have dined first, are ingested by flagellates and ciliates, and eaten by other zooplankton. There are periods during the summer when phytoplankton is particularly scarce, small larvae and other zooplankton at their greatest concentration, and many fragments of organic matter are suspended in the water. During these periods it is not obvious how the numerous zooplankton organisms obtain sufficient food unless this detritus is eaten. It has been observed that during such periods the faecal pellets change in colour from green to brown, suggesting that the diet contained a higher proportion of organic detritus (Mare, 1940).

In considering the effect of animals on plant production, it is axiomatic that the maximum possible fertility will coincide with a balanced population of carnivores, herbivores and plants. In the sea each occur patchily both in time and space, so a lack of balance is the usual condition. A departure from balance in any one of the three directions cannot persist, but will in time not only right itself but probably swing in the opposite direction.

VII. REGENERATION OF NUTRIENT SALTS

Productivity in the open oceans is usually controlled by the rate at which available nitrogen compounds and phosphates are supplied to the photosynthetic zone, either from the water below by vertical mixing or by regeneration *in situ* from living organisms which are eaten or die. The deep waters of the oceans hold a great store of these salts. In relatively shallow areas where the photosynthetic zone may extend in summer to a half or quarter of the way to the bottom the same statement applies. The nutrients in river water are mostly used up before the water runs into the sea in summer, at the time when the zone lacks these nutrients, and its effect in supplying them does not appear to extend far from the land.

Land drainage, on the other hand, may play an important role all the year round in supplying trace elements such as iron and manganese, both of which tend to 'pass out of circulation' as they are readily adsorbed on organic particles in the water.

Regeneration of nitrogen compounds which can be used by the phytoplankton, urea, uric acid, ammonia, nitrites and nitrates, and of phosphates, is of two kinds: *direct* and rapid when organisms are digested and the products of metabolism set free, *indirect* and slow when brought about by bacterial action and autolysis.

Concerning direct regeneration little is known; it should prove a fruitful subject for investigation since existing methods of analyses are likely to be sufficient. Gardiner's (1937) experiments, where *Calanus* fed on diatoms, show a rapid excretion of phosphorus as phosphate. This suggests that direct regeneration may return much of the grazed living matter as salts into the photosynthetic zone. Some at least will be returned to water lying below the zone, since most herbivores migrate downwards during the day.

The proportion of organic matter undergoing direct regeneration, both within and below the photosynthetic zone, to the proportion which undergoes indirect

regeneration, is quite unknown. There are, however, indications that the latter plays no inconsiderable part; sea water contains a material store of organic nitrogen compounds in solution (Krogh & Keys, 1934; Robinson & Wirth, 1934). Redfield *et al.* (1937) have followed the amount of organic phosphorus compounds in solution; this increased during the summer, reaching a concentration which averaged some 11 mg. P/m.³ in the upper layers and fell to some 2 mg. P/m.³ during the winter months.

This summer value exceeded the quantity of phosphorus ever found in the plankton and detritus present in a cubic metre. It may be concluded that during much of the year a rather considerable quantity of both nitrogen and phosphorus remains locked up in the form of dissolved organic matter.

The processes of indirect regeneration have received more attention from marine biologists. These processes break down to inorganic salts both organic matter in suspension and organic matter in solution; Redfield's investigation indicates that much of the phosphorus in seas of moderate depth is present as organic matter in solution during late summer.

It is simplest to deal first with the breakdown of organic matter in solution, of which least is known. Offshore sea water, freed from plankton, contains few bacteria, 5-200 per c.c. being usual, an average sample having some thirty species. When such water is put into a vessel, the number of bacteria soon increases to several hundred thousand per c.c. in the water and very many develop on the sides of the vessel, the number of species falling to nine or ten by the time the bacterial population has reached its maximum. Later the number of bacteria in the water declines and the number of species falls to four or five (ZoBell & Anderson, 1936). When plankton-free water is stored, Keys *et al.* (1935) have observed that the ammonia in it sometimes increases and sometimes decreases. Unless the water has been collected close to the bottom or near shore the nitrite and nitrate remain unchanged. The phosphate increases slowly during storage, this increase being sometimes preceded by a decrease, almost certainly due to the utilization of phosphate by bacteria during their rapid proliferation. The quantity of phosphate set free finally is, however, very small; only a small moiety of the phosphorus probably present as organic phosphate is liberated. In spite of the very great number of bacteria which develop in stored water, it seems that they are only capable of dealing with a part of the nitrogenous and phosphorus-containing organic matter in solution (Harvey, 1941). Waksman & Carey (1935) find that about half the organic matter in solution is decomposed by bacteria when clear water is stored.

These changes in the ammonia and phosphate content of the water may not be entirely brought about by bacteria. Kreps (1934) found that changes in ammonium and nitrate took place in inshore water which had passed a Seitz filter or been sterilized with mercuric chloride, and suggested that sea water, particularly water near the bottom where organic matter was decomposing, contains enzymes which bring about these changes. Keys *et al.* (1935) have observed changes in the ammonium content of water which had been sterilized with mercuric chloride; Newcombe & Brust (1940) have observed that saturating water with chloroform

reduces but does not stop phosphate being set free during storage. Cooper (1937) has examined changes which may take place between inorganic nitrogen compounds in the sea and their energy relationships.

Passing next to the indirect regeneration of inorganic nitrogen and phosphate from plankton organisms decomposing in sea water, Von Brand *et al.* (1937, 1939, 1940) have observed that both phytoplankton and zooplankton set free their nitrogen as ammonia, while later this changes to nitrite and finally to nitrate. The time taken depends upon temperature, a rise of 8° C. about halving the time, and upon the source of the sea water in which the plankton is suspended. They found that all the nitrogen set free was utilized if water was inseminated with a diatom and kept in the light; hence an aliquot quantity of phosphate was presumably formed at the same time. Cooper (1935) has followed the liberation of phosphate when plankton breaks down in sea water, this occurring more rapidly with zooplankton than with phytoplankton. Two out of four samples of water which had been freed from larger plankton organisms showed little change in phosphate content when stored in the dark for several months, yet, where animal plankton had been added to these waters, not only was all the phosphorus added in this form regenerated as phosphate but a considerable quantity in excess. He concluded that this excess was set free from organic compounds present in solution in the sea water. It seems reasonable to surmise that species of bacteria grew on the rich food provided by the animal plankton, which were able to utilize the organic compounds in solution but which died out when plankton-free water was stored.

Von Brand *et al.*, in their investigation of the nitrogen-containing salts set free by plankton decomposing in sea water, have also observed more nitrogen set free in these forms than was originally present in particulate matter.

The precursors of ammonia, organic nitrogen compounds, particularly those yielding albumenoid ammonia, occur in considerable quantities in solution. Robinson & Wirth (1934) find between 10 and 90 mg. of albumenoid nitrogen per m.³ in the Pacific in addition to more firmly bound organic nitrogen.

Ammonia is found in quantities ranging from 0 to 60 mg. of ammonium nitrogen per m.³, the distribution being irregular but with greater concentrations occurring in the upper layers (Wattenberg *et al.* 1930; Buch, 1932; Cooper, 1933). In the western basin of the Gulf of Maine, Redfield & Keys (1938) find material quantities throughout the water column in late autumn, but in May the photosynthetic zone was almost entirely depleted. A layer below the zone was left relatively rich in ammonia while the deep water below was almost free from ammonia. This intermediate layer was also relatively rich in nitrite.

The distribution of nitrite in deep temperate and tropic seas is peculiar. It is found in a layer below the photosynthetic zone, where decomposition of organic detritus has also reduced the oxygen content of the water. Thompson & Gilson (1937) in discussing this layer in the Indian Ocean suggest the formation of nitrite by bacterial reduction of nitrate; Rakestraw (1936) has stored samples of water from various depths and positions in the Atlantic in the dark, finding a small change in the nitrite content in some samples, but no change in samples freed from plankton.

It is a general observation that no change in either nitrate or nitrite occurs in plankton-free water during storage, even if enriched with ammonium, unless collected close to the bottom or near shore. This points to one of two conclusions, either the breakdown is due to bacteria attached to plankton organisms or the effective bacteria die out when plankton-free water is stored and other species proliferate. Otherwise, it is difficult to account for the distribution of breakdown products in the sea. Moreover, when plankton-rich waters are stored the breakdown products appear in the usual sequence: ammonia, nitrite and finally nitrate.

It is generally assumed that breakdown to orthophosphate is necessary before organic phosphorus compounds become available to marine plants. This is not the case with higher plants, Rogers *et al.* (1940) having observed that both phytin and lecithin are absorbed as such by their roots. With regards to nitrogen, complete mineralization is not essential; there is evidence that urea and uric acid can be utilized as such, while communities of phytoplankton diatoms with their associated bacteria rapidly break down some amino acids to ammonium (Harvey, 1940). These observations suggest that sea water may contain quite respectable quantities of available nitrogen in addition to nitrates, nitrites and the 5-30 mg./m.³ of ammonium nitrogen commonly found.

VIII. MAGNITUDE OF ANNUAL PRODUCTION

The first attempts to gain an idea of the annual production of organic matter by photosynthesis were made in the following manner from data obtained in the English Channel. The decrease in carbon dioxide and in phosphate in the water which took place between winter and early summer, when they were at a minimum, was found for a column of water below 1 sq. m. These quantities had been built up into plant material during this period of the year, when the standing crop is at its greatest. How large a proportion of the annual production occurs during this period is unknown. No account could be taken of the carbon dioxide absorbed from the atmosphere or produced by respiration of animals and bacteria, nor of the phosphate regenerated during this period and utilized again. Similar calculations of production were also made from the decrease in nitrate in the water, and here also no account could be taken of the quantity of ammonium regenerated and used again as such. The quantities calculated are minimum values for production during the half year. Since the original calculations were made, reliable analyses of diatom plankton have been published which give the ratio of carbon to nitrogen to phosphorus in them, and these have been used to recalculate from the data of the original observers.

Analyses of plankton diatoms

		C	N	P
Redfield (1934)	Bay of Fundy	100	18.2	1.36
	Nova Scotia	100	15.6	2.26
Waksman <i>et al.</i> (1937)		100	14.2	1.4
	Mean	100	16	1.67
Cooper (1937)	Mean ratio		16	1.96

The increase in dissolved oxygen in the sea over this period, due to photosynthesis, has been used for similar calculations of production. This also takes no account of loss of oxygen to the atmosphere nor of oxygen used in respiration by bacteria, animals and plants.

*Carbon produced by photosynthesis during half year below 1 sq. m.
minimal estimates*

	C g.
English Channel, from decrease in phosphate (Atkins, 1923)	53
carbon dioxide (Atkins, 1922)	84
carbon dioxide (Cooper, 1933)	101
phosphate (Cooper, 1933)	48
nitrate (Cooper, 1933)	39
English Channel, from increase in dissolved oxygen (Cooper, 1933)	60
Gulf of Maine, from decrease in phosphate (Riley, 1941)	40
Long Island Sound, from decrease in phosphate (Riley, 1941)	46

In arctic waters Kreps & Verjbinskaya (1932) have made similar minimal estimates of production from phosphate data collected at intervals throughout a year in the Barents Sea. Here the greater part of the production is compressed into a relatively short period, from early May to mid-August. During this period the phosphate in the water decreased by some 1.1 g. P below each square metre, indicating the production of 66 g. C/sq. m. During this period regeneration of phosphate was undoubtedly taking place, and the authors made a second estimate, allowing for this on the assumption that it was equal to the increase in phosphate which took place in the water during the subsequent 100 days. This allowance suggests that the consumption of phosphate during the growing period was some $1.1 + 0.45 = 1.55$ g. P, indicating the production of phytoplankton containing some 92 g. C below each square metre.

Another method of assessing annual production was evolved by Seiwel (1935) for waters of the tropical western North Atlantic, where a layer of oxygen-deficient water lies below the photosynthetic zone. He considered that this deficiency was due to the annual crop of organic matter being finally oxidized in this layer, and that the loss of oxygen was balanced by the supply from the water below and above brought about by turbulence. By assuming a value for the coefficient of eddy conductivity in this mid-water layer, the quantity of oxygen entering the layer was calculated. It was sufficient to oxidize 278 g. of organic carbon yearly below each square metre, and it was considered that this value represented the annual production of organic matter. The whole argument depends upon the magnitude of the assumed coefficient of eddy conductivity. The value for production is some four times greater than the minimal values calculated for the first half of the year in the English Channel which is an area relatively rich in plankton, and which would at first sight appear to have a very much greater annual production.

Gran (1927) and Marshall & Orr (1930) have suspended samples of sea water containing its natural flora and fauna in the sea, estimating the increase in oxygen

after a period of time. Similar samples in black bottles were suspended alongside as controls, the final difference in oxygen concentration in the light and dark bottles giving a measure of the *gross* production by photosynthesis. In both light and dark bottles oxygen was being used in respiration by plants, bacteria and animals, so the difference in concentration of oxygen attained by the end of the experiment between the light and dark samples was a measure of the total photosynthesis; part of the organic matter produced by the plants was being lost continuously in their processes of respiration; this does not enter into the final result. It is the *nett* production, that is, the gross production less respiratory losses, which is required to obtain the annual production.

Measurements of gross production made by suspending samples of unfiltered sea water in light and black bottles have been continued by Riley (1938, 1939, 1941). The quantities of carbon synthesized annually below a square metre were calculated from a knowledge of the depth of the photosynthetic zone, and experimental data obtained at intervals during a year. These calculated quantities range up to 1000 g. C/sq. m./year (Long Island Sound). From the quantity of chlorophyll contained in the plants, the respiratory losses were assessed, and these deducted from the gross production gave tentative values for the annual production of phytoplankton—the carbon produced which is in excess of respiratory requirements and can therefore be used in the production of new material. This novel method of arriving at the desired result is very dependent upon a correct assessment of respiratory losses. Riley has also used the difference in both phosphate and nitrate content which arises between the light and dark bottles as a measure of the nett production. The measure is not strictly direct, since the plant organisms continue photosynthesis when the supply of either is reduced, building up storage products and attaining a phosphorus or nitrogen deficiency (Ketchum, 1939*b*).

The magnitude of the tentative estimates based on these experimental methods are of interest.

Phytoplankton production (Riley, 1941)

		C/sq. m./year
		g.
Long Island Sound	O ₂ production	400-700
	P consumption	440-875
	N consumption	140-365
West Atlantic 23-41° N.		
Georges Bank, Gulf of Maine, O ₂ production		C/sq. m./day
		mg.
Jan.		—50
Mar.		190
Apr.		950
May		540
June		630
Sept.		140

Riley has concluded that no very great difference exists between the annual production in deep ocean waters of the tropics and in temperate latitudes, where insufficient light reduces growth during the winter. He points out that although phytoplankton is sparse in tropic waters, the low concentration of the breeding stock is partly counterbalanced by the great depth of the photosynthetic zone. The

concentration of nutrients is kept very low by rapid utilization, rather than by lack of available supply. Seiwel's calculation points in the same direction as Riley's conclusion, which is based on his own data of the rate of plant production in the Sargasso.

This view is opposed to the usual conception that deep tropical and subtropical oceans, away from land or upwelling water, are relatively barren wastes. Where comparable net hauls have been made vertically through the upper layers they have caught only a small fraction of the quantity of animals which are caught in temperate or high-latitude oceans. After making allowance for the seasonal changes in animal population in the latter areas, there is still a smaller average animal population in low latitudes. In these warmer seas the metabolic rate of the animals is doubtless greater—the temperature difference may be of the order of 20°C .—and a similar population would require a greater annual production of plant food. However, the extent of this extra nutritional requirement may not be great; Fox (1939) gives instances where the oxygen consumption of some species of marine animals is less at the same temperature for individuals which have been collected from a warmer environment.

IX. FLUCTUATIONS IN PRODUCTION FROM YEAR TO YEAR

Russell (1935) has taken comparable hauls with a net at weekly intervals throughout a number of years at a position in the English Channel. He observed a relation between the numbers of young fish which had been spawned in the summer and

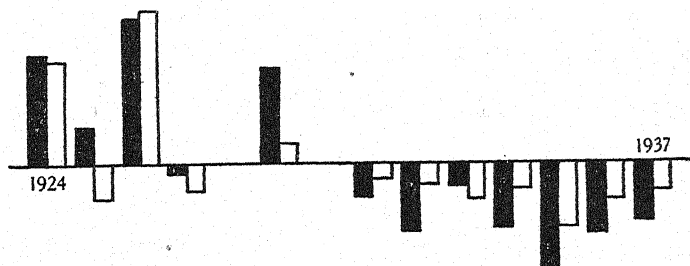


Fig. 1. Deviation from the mean winter maximum of phosphate and from the mean number of summer-spawned young fish for years between 1924 and 1937. The black columns above and below the horizontal line denote the extent to which the winter maximum of phosphate each year exceeded or fell short of the mean for the 12 years. The outlined blocks show the extent to which the average number of young fish caught per haul each year exceeded or fell short of the mean value for the 12 years.

survived their early larval stages and the 'winter maximum' of phosphate in the water at the beginning of the year. The figure taken from data published by Cooper (1938) shows this relation very clearly. The lower 'winter maxima' which occurred during the 1930's were also coincident with a change in the type of plankton (Russell, 1936), a *Sagitta setosa* community replacing a denser average population characterized by *S. elegans* (Fig. 1).

The marked difference in winter maxima of phosphate and general fertility between the 1920's and 1930's is not reflected in any well-marked change in the

temperature and salinity of the water during the transitional year or years. The concentration of a conservative constituent of the water, as the salinity, indicates its physical past history, whereas the winter maximum of phosphate indicates its biological history. Judging by these relations found by Russell, it also appears to indicate its biological future in this area.

X. MAGNITUDE OF THE STANDING CROP OF PLANTS

Early methods of sampling the standing crop of phytoplankton were to make comparable hauls with fine silk nets and count the organisms. Later, the volume of sea filtered by the nets was calculated and, more recently, it has been measured with an attached meter. In its various forms this technique allows a proportion of the smaller organisms to escape; the finest net has pores some $42 \times 50 \mu$ when the silk is wet and swollen, yet when it is drawn slowly through the sea it catches a considerable quantity of smaller organisms, many of which either occur in chains or have spines which entangle them. In some areas and at some times it undoubtedly catches the greater proportion of the plants, but in other areas or times it may give an entirely erroneous picture of the magnitude of the population. Another method has been to pump sea water through a hose from a series of depths and filter it; by this means of collection, counting the organisms and measuring the size of each species, comparable results have been arrived at. More recently samples of water obtained from a series of depths have either been centrifuged or have been poisoned, allowed to settle on the bottom of a cylinder, and counted from below with an inverted microscope. This sedimentation method has given considerably higher values than centrifuging and counting the cells in the deposit (Steemann Nielsen, 1933).

Such counts take a long time and the resulting tables of species and numbers can only be interpreted by an expert familiar with the various species as they occur in that area. The species vary greatly in size and in the proportion of plant tissue to sap within the cell. The size of individuals of a species varies (Wimpenny, 1936; Lucas, 1941; Gross, 1937); larger individuals of a diatom species are found in warmer than in colder seas.

A measure of the plants in a catch of mixed zooplankton and phytoplankton can be obtained by dissolving the plant pigments in acetone or alcohol and estimating either the mixed pigments or the chlorophyll. This technique has been used extensively; comparative estimates of net-caught plants have been made in the Barents Sea by Kreps & Verjbinskaya (1930, 1932), in the English Channel by Harvey *et al.* (1935), in the Indian Ocean by Thompson & Gilson (1937) and in the Antarctic by Hart (1941).

Plants caught by filtering 1-6 l. of sea through paper or a collodion membrane have been estimated by Riley (1938-41) in various areas off the American coast and by Krey (1939) in Kiel Bay.

The colour in the yellow-green extract can be expressed in arbitrary units, since the quality of the colour is nearly always the same. In the English Channel the unit

of plant pigments has been linked with the quantity of phosphorus in the plants, and in the Gulf of Maine with the quantity of organic matter in the plants (correlation coefficient 0.76).

The carbon content of the flora lying below each square metre can be calculated for these two areas.

At a position in the English Channel with a depth of 50 m., a particularly rich catch was made in March 1933 at the height of the spring outburst, when the netted plants contained some 7000 units of plant pigments per cubic metre of sea. This is commensurate with plants containing 0.025 g. of phosphorus and 1.5 g. of carbon below each square metre. The smallest catches were made in summer and mid-winter. In summer the water contains very small organisms which would pass through any net, but observations by the sedimentation method indicate that the summer flora is only a very small percentage of that in the spring.

In the Gulf of Maine, Riley (1941) concluded that the flora below a square metre contained some 2 g. of carbon when at its maximum and a negligibly small quantity in winter. He filtered samples of water from a series of depths through no. 2 Whatman paper, which was found to retain 90 % or more of the organisms.

While considering magnitudes it is of interest to make a similar calculation for the extremely fertile ocean around South Georgia. In the upper 50 m. the net-caught plants below a square metre had an average content of some $1\frac{1}{2}$ million units of plant pigments during the summer. If we assume the same ratio between plant pigments, phosphorus and carbon in these antarctic diatoms as have been found elsewhere, this indicates plants containing some 7.2 g. C/sq. m.

These values can only be considered as very rough estimates of the quantity of carbon in plant protoplasm and food reserves at the season when the plant population is at its height. As with the estimates of annual production, they are first attempts to deduce the quantity, in absolute units, of the food supply of the fauna.

XI. SOME NATURAL PHENOMENA

Present knowledge of the factors which control production is incomplete; vertical mixing by turbulence with its twofold effect and grazing by animals remain unmeasured; several chemical factors, which are as yet only suspect, may operate in nature and there may be other as yet unsuspected factors playing a major role at times in some areas.

Hence it is of interest to discuss a few phenomena which remain unexplained or of which an explanation lacks proof.

In temperate seas and in fresh water it is not uncommon for one species of diatom to multiply rapidly and build up a standing crop which persists for two or three weeks and then disappears, its place being taken by another species. Sometimes there is an interval with a very sparse flora, at other times the place of the first species may be taken by the second without break, the change commencing near the surface and extending downwards. The cause of this sequence of species remains entirely unexplained. It may be linked with a change in the specific gravity of the diatoms, sinking diatoms are found in the sea and a layer of diatoms is occasionally

found which have sunk below the photosynthetic zone. In cultures diatoms usually, but not always, remain suspended in the water while they are growing rapidly and sink when the growth rate falls off. This is not dependent upon the specific gravity of the water, since adding fresh water to the medium does not bring about either a rise or fall; the diatoms evidently possess a mechanism by which they can adjust their buoyancy to that of the medium. Factors likely to affect their buoyancy in nature have not been investigated; recent work by Gross (1940) on osmotic exchanges in a diatom suggest a line of attack.

Another phenomenon which seems to lack explanation is the time at which phytoplankton starts to increase rapidly at the beginning of the year. In temperate seas a considerable concentration of nitrates and phosphates, and presumably of all other constituents necessary for plant growth, is found in the upper layers before midwinter. The stage is set for rapid growth and the production of a large standing crop, but something less obvious than sufficient light seems to be necessary before the spring outburst of diatoms takes place. In the deep waters of the Gulf of Maine in latitude $41-43^{\circ}$ N. at $2-3^{\circ}$ C. it occurs no earlier than in latitude 63° N. in the deep water off the Norwegian coast at $4-5^{\circ}$ C., nor in the relatively shallow water of the English Channel at some 8° C. in latitude 50° N. In some parts of the Gulf it may occur no earlier than in water of the Barents Sea at -1° C. in latitude 73° N.

In the region of the Gulf of Maine considerable growth is recorded in December-January at Woods Hole and Cape Cod Bay, but in the deeper offshore waters this does not happen until March-April, there being as much as six weeks difference between various parts of the open Gulf (Bigelow *et al.* 1940). Over Georges Bank it occurs before it takes place in the deep water around it; Riley (1941) points out that loss of breeding stock in the photosynthetic zone will be greater during storms in deep water, since turbulence will carry some of the plants to greater depths than is possible over the bank. A similar explanation of growth taking place over the Faeroe Bank before it takes place in the surrounding deep water has been advanced by Steemann Nielsen (1935).

Off the Norwegian coast, Braarud & Klem (1931) observe the spring outburst to occur first in the sheltered waters of the fiords and in the deep water beyond the shelf which extends some 20 miles seaward. Over the shelf growth starts later.

In the Arctic, Kreps & Verjbinskaya (1930) have found a considerable growth of phytoplankton taking place early in May in Atlantic water at -1° C. in latitude 73° N., whereas farther south in water of $+1.5^{\circ}$ C. of arctic origin in latitude $70-71\frac{1}{2}^{\circ}$ N. growth did not start until later.

It is remarkable that the spring outburst was taking place in early May in 73° N., while Bigelow *et al.* (1940, p. 159) consider that it may be expected about the same time in the northern part of the western basin of the Gulf of Maine, in 43° N.

In the Southern Hemisphere, Dakin & Colefax (1940) note that the spring increase occurs at about the same time prior to midsummer off the east coast of Australia in 34° S. as off the Californian coast in 36° N. latitude.

Between latitudes 50 and 70° S. in the Antarctic, the standing crop increases rapidly to a maximum starting about 3 weeks before midsummer (mid-December)

in the more northerly latitudes and not before midsummer in the more southerly latitudes. However, off South Georgia and in the Scotia Sea the inception is some 3 weeks earlier than in the same latitude away from the influence of land (Hart, 1941).

These considerations suggest unrecognized factors controlling the inception of growth in some areas. The subsequent decline in the standing crop may be due to grazing; it occurs at a time when herbivores are increasing rapidly and is often associated with great numbers of faecal pellets in the water. Doubtless the intensity of grazing and the hindering effect of turbulence affect the inception of the spring outburst, but there is no evidence that they account for the anomalies which have been mentioned.

The production of plant life in South Atlantic waters has presented outstanding problems. Throughout the year the upper layers are well supplied with phosphates and nitrates in the southerly latitudes; the standing crop in summer varies enormously in different areas. There is no ascertained reason why the production should not be very much greater than it is.

A remarkably rich flora occurs in the deep ocean lying 20–120 miles off the island of South Georgia which rises steeply from the ocean floor; the standing crop in summer is some ten times greater than in the surrounding ocean, subject to similar hindering of growth by turbulence. In the Scotia Sea, fed by a current which has washed outlying islands of the Antarctic continent and passed over a submarine ridge, the standing crop is twice as great as elsewhere in corresponding latitudes. Hart (1941) attributes these heavy developments of plant life to a better supply of some constituent derived from land drainage, such as iron or manganese.

He also points out that both in these rich areas, and in those far from the influence of land drainage, the plants take out of the water many times more phosphate than is ever found present as phosphorus in the standing crop when it reaches its summer maximum, and considers that the latter is continuously subjected to very heavy grazing. In these seas control appears to be effected by (i) hindering of growth due to turbulence, (ii) grazing, (iii) lack of some constituent as iron or manganese supplied by land drainage, and (iv) short supply of silica on occasions. The first three could account for the observed distribution and quantities of phytoplankton.

XII. SUMMARY

This review deals with the production of plants in the sea, which is controlled by a mosaic of factors, many of which are interrelated. Observations are collected and discussed, which relate to (i) the compounds of nitrogen and phosphorus directly utilized by the phytoplankton, and their rate of supply to the photosynthetic zone; (ii) the depth of this zone, which is determined by the quantity of light energy entering the water daily and the transparency of the water; (iii) the influence of temperature, of light, and of the concentration of nutrient salts, upon the growth rate of some diatoms; (iv) the dual effect of turbulence which, on the one hand, carries nutrient salts from below into the photosynthetic zone and, on the other hand, carries plant organisms to depths below the zone and, in some areas, increases

the turbidity of the water thereby reducing the depth of the photosynthetic zone; (v) grazing by herbivores which deplete the breeding stock of plants but also excrete nutrient salts directly into the photosynthetic zone; (vi) the regeneration of nutrient salts by bacteria and enzymes; (vii) the supply of iron and other trace elements, of which manganese and divalent sulphur compounds are needed by some species of diatoms.

The supply of such minor constituents is thought to play a considerable role in the distribution of plant life in the southern South Atlantic, where there is a high concentration of nutrient salts in the waters throughout the year and great differences in the density of the flora between different areas.

Estimates of the magnitude of the annual production of plants below a square metre, arrived at by various indirect methods, are compared.

Fluctuations in the annual plant production from year to year in the English Channel, assessed from the quantities of phosphate present in the water during the winter, show a marked correlation with the density of the fauna.

Recent attempts to estimate the standing crop of phytoplankton in terms of food for animals are described, and tentative estimates of the carbon and phosphorus content of the standing crop below a square metre, when at its maximum in several seas, are compared.

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ON THE DEVELOPMENT OF FEATHERS

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GOOD reviews of the development of feathers from the morphological standpoint down to about 1930 may be found in the following publications: Davies (1889), Strong (1902), Stresemann (1927-34). These may serve as a point of departure for the morphology of feather development. The experimental analysis of feather development, which is of recent origin, has not been systematically reviewed previously. It will therefore be our main topic. On account of the various uses of feather development in biological research it becomes rather difficult to delimit the field of review. But development itself will be the principal subject, and the application of this knowledge, e.g. in endocrinology and genetics, secondary. The larger part of experimental work has been on various breeds of fowl, and the present account is so limited except where otherwise indicated.

I. GENERAL MORPHOLOGY OF FEATHER DEVELOPMENT

(1) *The origin of the feather papillae and tracts*

Feathers arise from superficial papillae consisting of a relatively massive mesodermal core in the corium, and a thin epidermal covering. The papillae make their appearance in certain definite arrangements as elevations on the surface in chick embryos between the sixth and ninth days of incubation. They are grouped in

correspondence with the definitive feather tracts, separated from the beginning by apteria. Within each tract the order of origin and the arrangement of the papillae is in a definite pattern. Before hatching, each papilla is sunk beneath the surface of the skin in a tubular follicle lined with epidermis and opening on the surface. The number of papillae in each tract is the same in the adult as in the embryo and each of them persists through life (Holmes, 1935). New papillae are not formed in the tracts after early embryonic development, unless in the case of inconspicuous filoplumes, though a few fluff feathers appear in the apteria.

The order of origin of the feather papillae in the tracts is significant not only for the arrangement of feathers in a given tract but also for their size and degree of asymmetry (Juhn & Fraps, 1934*d*; Fraps & Juhn, 1936*b*). Each tract has properties of an 'embryonic field' in which position is significant. Holmes (1935) states that

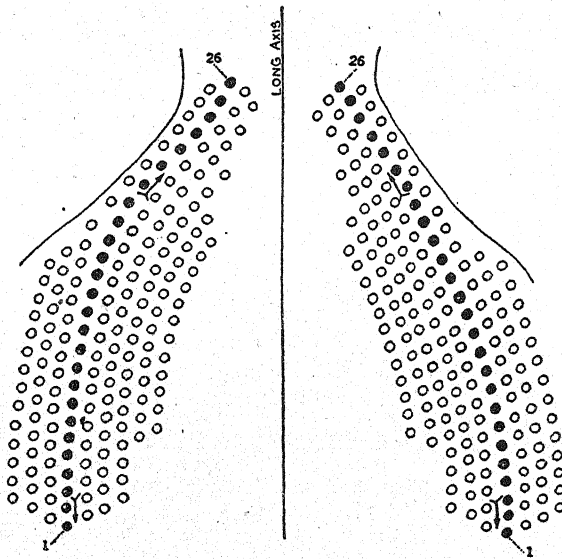


Fig. 1. Diagram of the breast tract. The black discs indicate the papillae of line of origin of the tract. Those enclosed by arrow bases are papillae arising simultaneously; the arrows give the direction of the extension of the line. The circles to the right and left of the line of origin are the lateral papillae arising in order. (After Holmes, 1935.)

'the first indication of a feather tract is a sub-epidermal condensation of cells in a ridge which usually has an antero-posterior direction in relation to the axis of the bird's body'. This breaks up into hillocks forming the first series of papillae. In the case of the breast tract, papillae of the second order arise on each side of the first series, alternating with them in position, presumably by lateral extensions of the original mesodermal ridge. Similarly papillae of third and later orders arise. The first series is also extended anteriorly and posteriorly, giving the definitive arrangement for the breast tract shown in Fig. 1. Similar principles apply to other feather tracts.

The plumage of birds in general is characterized by a succession of feather forms pertaining to definite stages of the life history. In all birds there is a sharp distinction

between the down feathers of nestlings (nessoptiles) and the feathers of the definitive plumage (teleoptiles), but intermediate types of plumage are distinguished in some birds, especially fowl. Development naturally varies in correspondence with the structural modifications. Development of the down feathers offers the greatest contrast to that of definitive feathers. Watterson has in the press (1942) the first comprehensive account of their development since Davies's paper (cf. also Watterson, 1941).

(2) *The development of the definitive feather*

Davies's paper (1889) is a really classical account of the development of feathers, on which all subsequent study of the subject has rested securely. Strong's paper (1902) was an important advance, especially with reference to pigmentation. Among

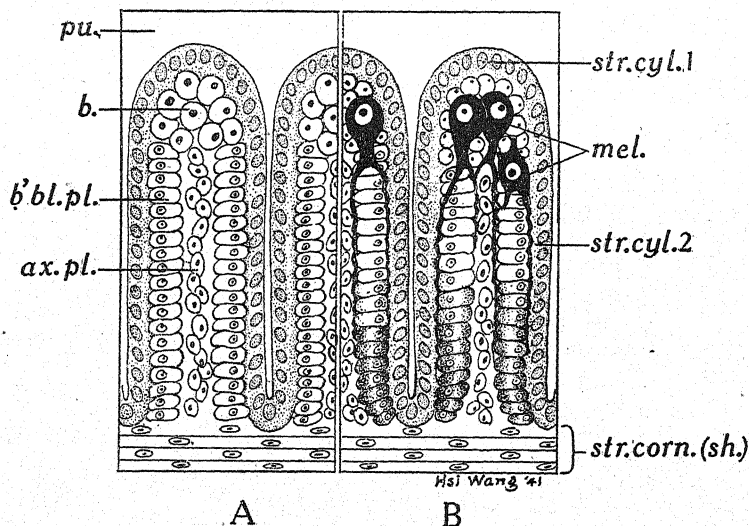


FIG. 2. Cellular composition of barb-forming ridges in transverse section from germs of breast feathers; A, of the White Leghorn; B, of the Brown Leghorn (capons): *ax.pl.* axial plate; *b.* cells of the barb-stem; *b'bl.pl.* barbule plate; *mel.* melanophores; *pu.* pulp of the feather cylinder; *str.cyl.* stratum cylindricum; 1, the part bounding the pulp; 2, the part between ridges; *str.corn.(sh.)*, the stratum corneum forming the sheath of the cylinder. Diagrammatic.

more recent studies, those of Kuhn (1932) and Greite (1934) are especially useful. There is not space in the present review for morphological details. For purposes of reference and terminology a single diagram (Fig. 2) of the structure and composition of a barb-forming ridge (cf. Fig. 4) must suffice.

A new feather arises from the papilla at the bottom of a follicle after natural moulting or after plucking of the feather whether completely formed or in process of regeneration. The presence of the feather in the follicle inhibits morphogenetic activity of the papilla always ready to be exercised. The papilla gives rise to a feather cylinder composed of an ectodermal wall derived from the ectoderm of the papilla and a mesodermal core (the pulp) derived from the mesoderm. The wall retains its cylindrical form within a sheath some distance beyond the apex of the pulp and above this the finished feather unfolds.

The ectodermal wall consists of three layers, an external one (*stratum corneum*) composed of keratin forming the protecting sheath of the developing feather, a thicker middle layer (*stratum intermedium*) from which the feather proper is derived, and an internal layer (*stratum cylindricum*) next to the pulp and regulating its functions. The external and internal layers are analogous to embryonic membranes. The three ectodermal layers are derived from a thick ring of embryonic cells, the collar (Lillie & Juhn, 1932), bounding the umbilicus. Differentiation progresses from the collar in an apical direction. The relations of these parts in the mid-process of regeneration of a breast feather of a Leghorn fowl is shown in Fig. 3.

The rachis of the feather develops along the outer (dorsal) surface of the posteriorly inclined cylinder. The opposite side of the cylinder next to the surface of the body is denominated ventral. If a proximal portion of the cylinder is split longitudinally midway between dorsal and ventral surfaces, spread flat and mounted with the inner surface exposed (Fig. 4), a very instructive view of the arrangement of the developing parts is obtained. The rachis is strictly parallel to the axis of the cylinder, but the barb-ridges, which are attached to the sheath (cf. Fig. 2), are inclined on each side with reference to the rachis; if followed beyond the limits shown in the figure, the apex of each barb is found to lie next to the midventral line; thus in the intact cylinder each finished barb describes a half-spiral in the wall.

The apexes of the barbs of the two sides form a configuration next to the collar known as the ventral triangle (Lillie & Juhn, 1932) situated in the midventral line in the case of symmetrical feathers, but more or less excentrically in the case of feathers with half-vanes of unequal width. Each barb apex, formed first at one or the other side of the ventral triangle, is destined to grow at its base to its full length, and, as it grows, to move dorsally across the collar to its junction with the rachis where its growth terminates.

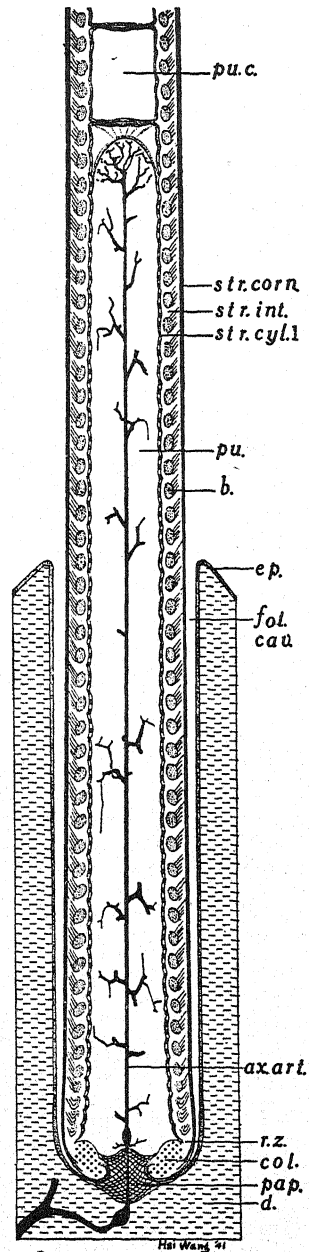


Fig. 3. Diagram of the feather cylinder in longitudinal section. White Leghorn capon: *ax.art.* axial artery in the pulp; *b.* barbs; *col.* collar; *d.* derma; *ep.* epidermis; *fol.cav.* cavity of the follicle; *pap.* papilla; *pu.* pulp; *pu.c.* pulp cap; *r.z.* ramogenous zone; *str.corn.* stratum corneum; *str.cyl.l.* stratum cylindricum; *str.int.* stratum intermedium.

There are two components of growth in each barb: (1) its *axial growth*, defined as the distance from its apex to the collar along a 'generator' of the cylinder, thus parallel to the axis of the cylinder and to the rhachis, and (2) its *tangential growth*, which is an added amount necessary to compensate for the tangential movement of the growing barb from its ventral point of origin to the rhachis. As axial growth is equal at all transverse levels of the cylinder, each barb must grow at a slightly greater rate than the rhachis.

The seriation of the barbs on the collar (Fig. 4) is in the order of increasing age from ventral to dorsal, and in any simultaneous complement the growing region in the same seriation represents successively more basal levels of barbs. Thus in the finished feather there are two time sequences, viz. from apex to base of the feather, and from the margins to the rhachis, with special reference to the barbs.

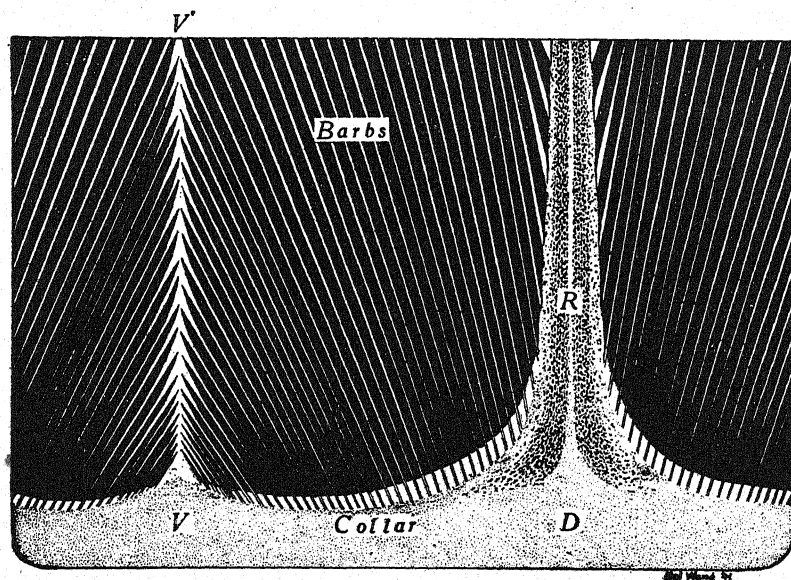


Fig. 4. Interior view of the base of a flattened split cylinder of a breast feather of a Barred Rock capon. Diagrammatic; less than half of the barb ridges represented. *D*, dorsal side of the collar with rhachis (*R*); *V*, ventral; *V-V'*, mid-ventral line of the cylinder. The barbs, black from melanophores, terminate next the collar in portions in which melanophores have not yet developed.

(3) *Correlation of development and structure: isochrones*

These arrangements enable us to correlate feather development with feather structure and pattern according to two principles which we shall designate as equal axial growth rates and the principle of isochrones. By equal axial growth rates is meant that all parts of the feather cylinder, including the pulp, grow axially at the same rate (cf. Lillie, 1940). The term 'isochrone' was introduced by Hardesty (1933) and defined by her as a line uniting 'the locus of points that were formed at the same time in the feather germ'. This corresponds to Lillie & Juhn's principle of loci of 'equal physiological age' (1932, p. 150), in the series of barbs simultaneously present on the collar (a 'barb complement') at any time in development

(Fig. 4). It thus defines *inter alia* points of simultaneous reaction to any factors affecting the growth and differentiation of the feather, such as nutrition, genetic factors, hormones, etc. As the reactive region of any barb is close to the collar owing to the rapid process of differentiation above it, the primary isochrone is close to and parallel with the collar ('collar isochrone'), but there is the possibility of secondary isochrones, diverging slightly from the collar isochrone, if points of simultaneous reaction to a given factor should not be parallel. This appears to be the case in the determination of certain pigment characters, where the isochrone bends up near the ventral triangle (Juhn & Fraps, 1936).

Axial growth and isochrones may be projected on the feather itself. This has been done by Fraps & Juhn in a very beautiful analysis (1936*b*). In this way any pattern in the definitive feather may be related to the corresponding developmental events in the immediate neighbourhood of the collar. The projection of equal growth increments and of the collar isochrone on the definitive feather, when the barbs are arranged at right angles to the shaft, furnish barb-shaft co-ordinates from which the order of events in the germ and the effects of altered physiological conditions may be plotted. The original paper should be consulted for methods and argument.

(4) *Origin of the rhachis and barbs*

A discussion of the developmental origin of the rhachis is important for the interpretation of experiments. Most students of feather development have followed Davies's account (1889) in essentials (cf. Strong, 1902*a*; Greite, 1934; Hosker, 1936). According to these accounts, the rhachis is formed from the barbs or from the same kind of embryonic material. Davies stated that the apex of the shaft is formed by fusion of barb ridges in the centre of the dorsal side of the cylinder and is continued posteriorly by fusion of the bases of later formed barbs with the central ridge. Lillie & Juhn (1932) were the first to maintain that rhachis and barbs are of independent origin. Their original view that the entire shaft was formed by concrescence of lateral halves of the collar was subsequently modified by them (1938), and they made a distinction between the longitudinal median portion of the shaft which arises independently and the lateral surfaces which they derived from the bases of the barbs and ramogenous material between them.

Lillie & Wang (1941) carried the analysis farther and showed that the lateral components of the rhachis are formed by special modifications of the bases of the barbs, 'barb petioles' (Fig. 4), which are flattened to conform to the shape of the central rhachis and bound together longitudinally by keratin fibres arising in each petiole and serially interlaced. It is possible to separate the central and lateral components of the rhachis by softening the feather in 5% potassium hydroxide after incising the rhachis a short distance longitudinally. The barb petioles can then be detached from the central rhachis and from one another. Each carries a continuation of the series of proximal barbules as far as the insertion of the next barb; thus the barbules on the rhachis between barb insertions really belong to the barbs. The central portion of the rhachis is derived from an independent primordium in the centre of the collar dorsally. It is prolonged apically to a minute extent by the

fused bases of one or two apical barbs on each side; thus the apical termination of the central rhachis proper is proximal to these barbs. The independent and bilateral origin of the central rhachis was supported by experiments described later in this article.

The barbs are formed individually from ramogenous material situated in the apical margin of the collar; about twenty-five are formed first simultaneously as ridges on each side of the dorsal surface (Lillie & Juhn, 1932). As development proceeds, the locus of origin of new barbs on each side is gradually transferred to the ventral triangle as already described (cf. Fig. 4).

The principal point to be emphasized with reference to the relations of the central rhachis and barbs is their entirely independent origin and the secondary nature of their association. The factors that are concerned in the determination of their union into the individualized form of the feather are discussed in the experimental section.

(5) *The after-feather*

Hosker (1936) was the first to consider the development of the after-feather. She described only a relatively late stage in its development and hence missed the critical early stages necessary for understanding the origin. Lillie & Juhn (1938) made a detailed study of the subject and found that the development is associated with a division of the ventral triangle in a late stage of feather development. Each daughter triangle, like the original ventral triangle, produces barbs on both sides; those on adjacent sides of the two triangles form barbs of the after-feather, and those on the opposite sides barbs of the main feather. A hyporhachis arises independently in the centre of the group of the after-barbs which grow in a strictly axial direction at first, but with the formation of the hyporhachis there begin tangential movements of the after-barbs relative to it. The formation of the after-feather is thus essentially a phenomenon of twinning, with certain characteristic resemblances of pattern to the main feather at corresponding levels and with reversal of sidedness of asymmetrical patterns due to the apposition of the ventral surfaces of the two feathers.

(6) *The pulp of growing feathers*

The growth, blood supply and functions of the pulp of growing feathers has also recently been studied in detail (Lillie, 1940). In connexion with this study the development of the *stratum cylindricum* and its role in the formation of the pulp membrane, the pulp caps and the barb septa were considered.

II. EXPERIMENTAL ANALYSIS OF FEATHER DEVELOPMENT

(1) *Experimental morphogenesis of the feather*

(a) *Operations on the papilla.*

The experiments of Lillie & Wang (1941) are the only ones in which methods of isolation of parts, transplantation and recombination, such as are current in experimental embryology, were applied to the development of the feather by opera-

tions on the papilla. It had not been realized previously that the feather papilla is the sole basis of development and that it is accessible to operation. Experiments were made on different feather tracts of Brown and White Leghorn fowls, but more especially on the breast tract.

The bird is anaesthetized, selected feathers plucked, and a papilla exposed by slitting a follicle to its base (cf. Fig. 3). Papillae of breast feathers in process of regeneration ('active papillae') are usually selected as possessing certain advantages. Such a papilla is about 1 mm. long and wide. It consists of a massive mesodermal core covered by a single layer of ectodermal cells except at its extreme apex. Inactive papillae are somewhat smaller and completely covered by ectoderm. The operation is made under a binocular microscope with fine iridectomy scissors.

If the papilla is completely removed, the follicle from which it came never regenerates a feather. On the other hand, the isolated papilla may be transplanted to another site, and there it regenerates into a feather of its own kind. If a papilla, dissected completely free from all attachments, is rotated through 90° or 180° around its own axis and re-implanted, the feather which develops from it is similarly disoriented, in the latter case upside down with the dorsal surface next the skin. It was thus demonstrated by many experiments that feather papillae possess a fixed innate bilateral organization. A papilla of the breast tract transplanted to an empty saddle follicle develops into a breast feather in the saddle. In the reciprocal experiment, a saddle feather forms in the breast. The papilla is thus shown to possess the specific potencies of its own tract; it is tract-specific. Experiments on transplantation of skin from one feather tract to another had previously shown that the transplants develop feathers of the type of the tract of origin (Danforth, 1929, 1937). The present experiments show that the specificity resides in the papilla, not in the transplant as a whole.

The transplantation experiments described were autoplasmic with a very high percentage of success. When made between breeds (unpublished experiments) the percentage of success is much lower, but other interesting problems arise.

Defect and isolation experiments were performed on papillae, with the following general results:

(1) The isolated dorsal half, or even one-quarter, of a papilla will develop into a normal feather. But the ventral half when isolated never does so: barbs develop from it but the rhachis is characteristically absent, and the barbs develop vertically, not obliquely, in the wall of the cylinder; they continue to grow indefinitely during the whole period of regeneration, and each forms an apical vane-like segment and a fluffy basal segment. The resulting feather is a tuft of barbs. *Capacity for forming a rhachis is limited to the dorsal half of the papilla. The rhachis is of different origin from the barbs.* Another common product of 'ventral' halves is the 'half-vane' feather, with a typical half of the vane on either the right or left side and the other half-vane represented by tuft barbs. This is presumed to be due to accidental inclusion of the right or left margin of the dorsal half of the papilla in the isolate.

(2) A lateral half or even third of the papilla is also capable of forming a normal feather. Thus the 'rhachis field' extends over the entire dorsal surface. It is possible

to produce similar twin feathers in one follicle by sagittal bisection of the papilla; but as the halves remaining in contact tend to heal together, complete twins are produced relatively rarely, and the more common result is a greater or lesser degree of apical twinning.

(3) The basal half of a papilla from which the apical half has been removed produces a feather that is normal except for about 10% of its length at the apex where various abnormalities occur. The result is entirely different from what would be produced by amputation of the apical tenth of a normal feather. The apex is not pre-localized in the papilla, but the removal of the apical half of the papilla produces readily understood abnormalities of the developmental processes.

It is possible to produce tract-chimaera feathers by combining contralateral halves of a breast and a saddle papilla respectively in the follicle belonging to one of them. The result is especially striking in the Brown Leghorn male or capon, in which breast and saddle feathers differ in form, colour and distribution of barbules. The chimaerae are perfectly individualized, intermediate in form, but with one side of typical breast colour and structure and the other equally typically saddle.

Operated papillae continue to produce the same types of abnormal feathers during at least several regenerations. There appears to be but little capacity for regulation.

In the defect and isolation experiments many abnormalities of the barbs are produced. These may be reduced to a few categories:

(1) Barbs that grow vertically and continuously; this happens when the rhachis is absent.

(2) Barbs that branch apically, sometimes very profusely ('branched').

(3) Barbs that divide in a basal direction, often repeatedly ('united').

(4) Barbs that branch from an intermediate fusion of united barbs ('bundled').

(5) Barbs united by their apexes to the rhachis ('reversed'); these occur only in half-vane feathers on the defective side of the half-rhachis.

These experiments demonstrate that the papilla controls the orientation and general development of the feather. It is believed that the organizing power resides at first in the mesoderm of the papilla and is exercised on the ectodermal covering. That the latter becomes a self-differentiating system very early in development is indicated by the experiments of Woitkewitsch (1936), who found that if regenerating wing coverts of 10-13 days in pigeons were pulled out as far as the mouth of the follicle, rotated 180° on their long axis and then replaced, those that resumed growth became upside-down feathers. The papilla was presumably not disoriented by this operation; but the collar was, and it now controlled the orientation of the feather, which was previously under control of the papilla.

The dorsal half of the dermal papilla induces the location of a dorsal field in the ectoderm within which the primordium of the rhachis arises, a capacity entirely lacking in the ventral half. With the induction of the dorsal field, the morphogenetic activity of the papilla appears to be accomplished; though its activity in producing the nutritive pulp persists throughout regeneration (Lillie, 1940).

Barbs arise and grow independently of the rhachis, but the order of their origin,

their tangential movements, and the sidedness of the barbules are regulated by the rhachis. Much of the evidence for these conclusions cannot be presented for lack of space. But the entire absence of tangential movements in the absence of the rhachis deserves emphasis. Irregularities in order of origin may be demonstrated by irregularities of lengths of barbs which indicate irregularity of origin according to the principle of equal axial growth rates. Under such conditions of irregularity of origin, crowded barb bases may fuse, thus producing branching of barbs; isolated bases may divide producing union as previously defined; alternate fusion and division of barb bases will produce bundling. For the theory of reversed barbs and reversal of sidedness of barbules reference must be made to the original paper.

(b) *The origin and behaviour of the melanophores.*

This subject has entered on a new phase of development with the experimental studies of Willier and his associates (cf. also Dorris, 1936, 1938, 1939; and Eastlick, 1938, 1939*a, b*). Previous work had been entirely descriptive and will not be reviewed here (cf. especially Strong, 1902*a*; and Greite, 1934).

Following the clue that the melanophores of Amphibia are derived from cells of the neural crest, Dorris (1936, 1938, 1939) was the first to show by explantation and transplantation experiments that the neural crest of embryos of chickens produces an abundance of melanophores (cf. also Eastlick, 1939*a, b*). In transplantation experiments portions of the neural crest of donor embryos between 3 and 10 somite stages were inserted within the mesenchyme of the hind-limb bud of host embryos of 72 hr. incubation for the most part. When the donor was from a black or red breed, similarly coloured melanophores developed around the site of implantation. The reciprocal transplants, from white donor to pigmented host, sometimes also produced unpigmented (donor-coloured) patches of feathers in the host. By a more elaborate series of experiments Ris (1941) succeeded in demonstrating that probably all melanophores of the bird are derived from the neural crest.

The paths and the rate of migration of melanoblasts to their definitive locations may be determined quite accurately by transplantation experiments. Thus Willier & Rawles (1940) showed that they are present in head and trunk ectoderm and mesoderm after 62 hr. incubation, but are not present in the wing bud until about 80 hr., nor in the leg bud until after 96 hr. (cf. Watterson, 1938; Ris, 1941).

Willier *et al.* (1937) introduced the technique of transplanting small pieces of skin between embryos of pigmented and unpigmented breeds of chickens. Using donors and hosts of 75 hr. incubation, they grafted pieces of skin from the head (about 0.5-1 mm. in area), from which most, if not all, of the mesoderm had been removed, to small incisions at the base of the leg or wing bud. When the donor was a black or a red breed, and the host white, they found on the fifteenth day of incubation patches of donor-coloured down too large to be explained by mere growth in area of the graft.

Willier & Rawles (1940) followed the subject much farther. They demonstrated that grafts of pure mesoderm might produce identical results (cf. Watterson, 1938) and that donor ectoderm disintegrates in the mesoderm of the host, leaving no

coherent traces. The affected feathers are accordingly formed by host ectoderm; when the hosts are hatched and reared these feathers without exception possess the forms and rates of growth of feathers in the same tracts and position within any tract, as in homologous locations of control host birds. They are, however, invariably of donor colour and pattern. The latter condition usually persisted in juvenile plumage and then gradually disappeared and was replaced by host-coloured plumage.

That colour and pattern of such affected feathers are due to some influence arising from the implant of donor tissue is obvious. That this influence proceeds from donor melanoblasts is derived from the following considerations: (1) Positive results are obtained only from grafts of proper age with reference to location as mentioned above, i.e. after melanoblasts have reached the site; (2) The influence spreads from the site of implantation in a manner identical with the known migratory behaviour of melanoblasts; (3) The melanin granules of donor-coloured feathers are of the shape, size and colour of those of donor breeds, each of which possesses distinctive characteristics. These evidences were also supported by a variety of secondary considerations.

The above analysis furnishes us with means for investigating the role of melanophores in the coloration and pattern of feathers. The correctness of the analysis is supported by evidence from other experiments. Among the most remarkable are those of Rawles (1938, 1939) who used robin embryos as donors to the wing buds of 72 hr. White Leghorn embryos as hosts. At hatching, robin-coloured areas covering part or all of the operated wing and even adjacent areas were found to contain melanin granules identical in shape, size and colour with robin controls. The robin colour was finally replaced by the white host colour soon after sexual maturity. Danforth (1929) obtained 'mosaic feathers' at the margin of grafts of skin transplants between homologous feather tracts of different breeds of fowls (cf. Danforth & Foster, 1929). In a later paper (1937) he suggested that migration of breed-different chromatophores might have been involved. Lillie & Wang (1941 and unpublished results) exchanged papillae of breast feathers between Barred Rock and White Leghorn fowl and obtained barred feathers from White Leghorn on Barred Rock and vice versa. The source of the melanophores is the host in Danforth's and Lillie & Wang's cases, being thus the reverse of Willier & Rawles's experiments, but the principle involved remains the same.

The most remarkable fact that emerges from these experiments is that melanophores produce not only their specific colour, but also their specific pattern in feathers of breeds possessing different colour and pattern. It cannot be supposed that their migrations and arrangements to produce pattern are autonomous; the explanation must be sought in certain principles of development to which the melanophores react.

Willier (1941) brings out the essential features of the problem: in the first place, the genotypic composition of the donor melanophores is the controlling factor in both colour and pattern. Thus not only do melanophores from a one-coloured donor (e.g. Black or Buff Minorca, White Silkie) produce similar one-coloured feathers on the host, and melanophores from parti-coloured donors (e.g. Barred, Brown

Leghorn, guinea-fowl or robin) produce donor-like parti-coloured feathers on any host, but also sex-linked differences in pattern from male or female donors of melanophores are expressed in host feathers (for instance, the greater width of black bands in female Barred Plymouth Rock as contrasted with the male).

This being the case, the conclusion is obvious that some physiological rhythm in the feather germ in the White Leghorn must furnish a stimulus to which the barred melanophore reacts, to use barring as a special example, and the same would be true of other host breeds. The generalization would be that physiological rhythms corresponding to barring occur in all breeds tested and that only genotypically barred melanophores react positively to them. Among common rhythms there might be some that produce longitudinal patterns as well; this would help to explain to some extent the transmission of guinea-fowl patterns by guinea-fowl melanophores. The hosts used in these experiments are exclusively fowl, which on account of their common origin may have preserved certain fundamental rhythms of development in common. As Willier notes (1941), 'the validity of this hypothesis may be tested by grafting barred melanophores to birds having a physiological rhythm of a different order from that occurring in the common fowl'.

But in addition to such general rhythms which might be common to all breeds of fowl, the individual feather germ controls the special type of barred pattern produced, to use this again as an example. For instance, in White Leghorn hosts the barred melanophore produces broad, ill-defined black bars in the rapidly growing flight feathers, and relatively narrower, sharply defined black bars in the more slowly growing breast feathers.

In general, barred melanophores in other fowl hosts tend to behave much as they do in the donor breed in accordance with specific rates of growth and other physiological properties in various tracts, such as Montalenti (1934) has described.

There is thus constant reaction between melanophores and the feather germ influenced by extrinsic physiological conditions that may affect directly either one or both. From intrinsic properties of the melanophores on the one hand, of the germ on the other, and the reaction of both to extrinsic conditions, we may hope to learn much more about feather development.

Finally, we may note that intrinsic properties of melanophores have been studied in explants *in vitro* by Hamilton (1940*a, b*) with some results applicable to their behaviour in the developing feather. He found that skin of embryos of white breeds produces melanophores in tissue culture, but that they have a very low viability as compared with those of pigmented breeds; melanophores of recessive white breeds have an even lower viability than those of the dominant White Leghorn. Thus such melanophores die at a low level in the feather germ before they can deposit pigment in barb or barbule cells. The red melanophores of red breeds of fowl require the addition of sex hormones to the culture medium to develop freely. On this basis a distinction may be made between 'independent' and 'dependent' behaviour of melanophores.

(2) *Reactivity of the feather germ and its gradients*

The growing feather is a very sensitive indicator of the physiological condition of the bird (cf. Kuhn, 1932), and conversely, the nature of the reactions of the germ disclose important principles of development. The finished feather records permanently in its colour, pattern and structure the results of fluctuations of condition whether produced by experiments or otherwise. There is no more convenient or accurate record than the feather once one has learned how to identify the locus of reaction in the germ with the result in the finished feather; in short, to read its autobiography.

Reactions are confined to a narrow transverse zone immediately adjacent to the collar. Above this differentiation of the cells, with keratinization, sets a limit to alterations in morphogenesis. Within this zone, however, distinction may be made between a most basal totipotent region, and a region of determination within which, by interpretation of the induced patterns, slightly different levels for activation of chromatophores and of barbule cells, for instance, are to be inferred. As we are concerned in this article with the analysis of morphogenesis, we shall make no systematic examination of reactive agents. Instead, we shall base our account on the principles of development involved in known reactions.

(a) *Axial growth rates and reaction.*

Juhn & Gustavson (1930a) were the first to discover the feminizing effect of female hormone on growing feathers of the breast and dorsal tracts of Brown Leghorn capons and to use the reaction as a quick indicator (Juhn & Gustavson, 1930b; cf. also Juhn *et al.* 1930). They found that it was possible to induce hen feathering in the saddle tract of capons with a relatively low concentration of the hormone that did not feminize growing feathers of the breast. Higher concentrations produced feminization in both tracts. They concluded provisionally that the difference in threshold is a function of the marked difference in growth rate of the feathers of the two tracts (Fig. 5), the higher rate (breast) requiring a higher concentration.

The same authors (1931), with the collaboration of Faulkner, then made a special study of this correlation. By comparing the course of absorption and excretion of different doses of hormone administered at regularly spaced intervals of 2-3 days with the varying degrees of appearance and disappearance of the feminizing reaction in feathers of different growth rates, they were able to make very exact correlations between growth rates and threshold, not only between different tracts but within a single tract (breast).

Without disputing the correlation between threshold of response and rate of growth in Brown Leghorn capons as between different feather tracts, e.g. breast and saddle, which they did not examine independently, Greenwood & Blyth (1935) concluded from the results of extensive and careful study that the same principle was not adequate by itself to explain variations in the amount of reaction of different feathers in the breast tract alone. They found that age of the bird and the place of

reaction with reference to the long axis of the feather must also be taken into account, together with genetic variations in the population.

Greenwood & Blyth (1935) and 'Espinasse (1939) have shown also that 'intra-dermal' injections of minute quantities of oestrone (0.025-0.15 mg., insufficient to produce a positive result when injected intramuscularly) within a patch of plucked feathers in the breast of Brown Leghorn capons may produce asymmetrical markings in the vanes of the regenerating feathers which bear more or less determinate rela-

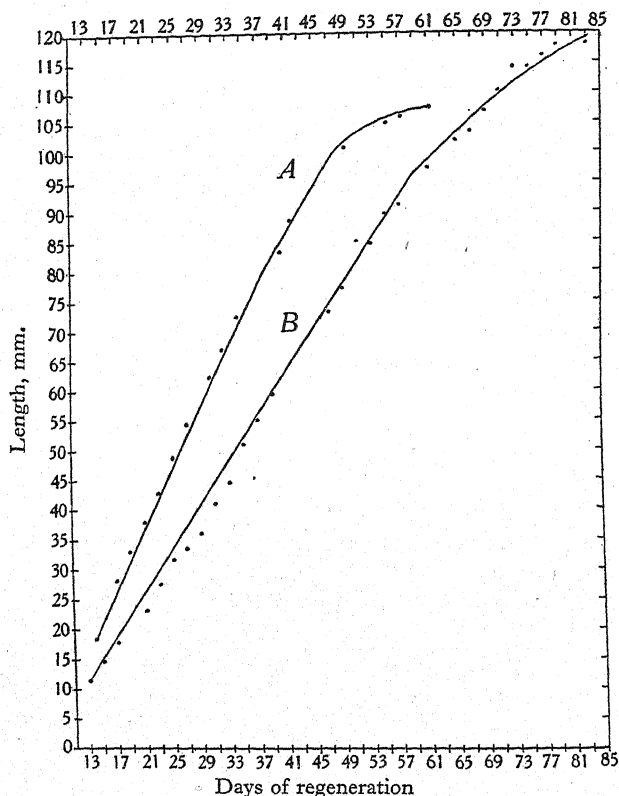


Fig. 5. Curves of growth in length of regenerating feathers. A, from the posterior region of the breast of a White Leghorn cock; B, from the saddle of a Brown Leghorn capon. The breast feather grows more rapidly than the saddle feather and its growth terminates earlier.

tions to the position of the intradermal site of injection. Presumably, as suggested by Greenwood & Blyth, the oestrone is distributed by diffusion through the tissues in this case to a greater extent than by the blood stream. If this is so, the results bear no relation to the theory of field functions as developed by Juhn & Fraps (1934, 1936).

The principle of a causal relation between growth rate of feathers and threshold of reaction has since been applied in a variety of ways; by Montalenti (1934), for instance, in interpretation of tract differences in barred patterns, and by Willier & Rawles (1940) and Willier (1941) in interpretation of patterns in feathers with foreign melanophores.

Pézaré (1922*a, b*) applied the idea of 'differential thresholds' to the explanation of gynandromorph plumage in fowl (cf. also Pézaré *et al.* 1926). However, he used only methods of ablation of gonads, either partial or complete, which were the only methods available at that time, and made no correlation with differential rates of growth. Subsequently Lillie (1931) presented a theory of bilateral gynandromorphism in the plumage of exceptional female birds based on the relation of differential reaction to the female hormone on the two sides of the body, one of which is hypertrophied and was presumed to possess a higher rate of growth. The contrary theory of somatic disjunction of sex chromosomes maintained by Bond (1913) and Goldschmidt (1931) and favoured by others (cf. Hutt, 1937) does not appear to apply very well to fowl.

The curves of growth represented in Fig. 5 are based on successive measurements at intervals of 24 hr. or more. Lillie & Wang (1940) have shown that the diurnal increments are not regularly accumulated, but that the rate of growth during a 24 hr. period exhibits constant fluctuations characterized by a very low rate during part of the night. This parallels other daily periodicities of functions in fowls, such as basal metabolism and body temperature. They suggested a correlation of the diurnal curve of growth with the occurrence of 'fundamental bars' in feathers, believed by Riddle (1907, 1908) to represent each a single day of growth.

(b) *Transverse gradients of threshold*

A very important principle with reference to the zone of reaction concerns gradients of threshold extending between dorsal and ventral limits on each side of the germ. This subject was first studied by Lillie & Juhn (1932), who found a gradient of threshold of reaction to female hormone and to thyroxin in the collar of regenerating feathers of Brown Leghorn capons. There is a gradual rise of concentration of reagents required for reaction of barbs from dorsal (next to the rhachis) to ventral positions (cf. Fig. 4). The consequence of this is that the reaction will show only next to the rhachis on each side with the lowest effective concentration. As concentration rises, the reaction appears at successively more ventral levels on each side until the ventral triangle is reached. The time after injection required for expression of reaction (*rate*) is inversely proportional to threshold, so that it is least ventrally and greatest dorsally.

Inasmuch as by the method of subcutaneous injection of the hormone there is a gradual rise of concentration at the site of reaction during the period of absorption, and a fall during the period of excretion, whenever the amount injected results in an increase of concentration above all threshold requirements, the reaction, combining threshold and rate, will leave a transverse bar of substantially uniform width across the vane of the feather. Such bars can be produced readily in the black breast feathers of Brown Leghorn capons by female hormone which produces the buff or salmon colour of the female feather. By interrupted injections spaced at intervals of several days such bars may be repeated in the same growing feather.

The long, lacy saddle feathers of the Brown Leghorn capon are especially well adapted to demonstrate the principle of gradients of threshold by their reaction to

thyroxin. Single injections of aqueous solutions of crystalline thyroxin into Brown Leghorn capons of about 1800 g. weight induce patterns in growing feathers of the saddle and neck which may be analysed by reactions in the germ adjacent to the collar. Single injections of solutions of thyroxin equivalent to 0.5, 1.0, 1.5, 5 and 10 mg. produce patterns varying according to amount; more than 10 mg. tends to induce moulting. The elements of the reaction concern widening of the extremely narrow axial web by formation of barbules and of melanophores which are absent in the lacy, barbule-free, more peripheral area of the vane of the normal feather. The contrast between the webbed black reaction mark and the normal orange lacy region of saddle feathers is striking. Similar reactions also may be produced in the neck hackles.

The duration of the reaction, occupying approximately 5 days with lower doses, is divided into a period of rising concentration and corresponding extent of reaction due to absorption, and a similar period of diminishing concentration due to excretion. The black mark produced by 0.5 mg. is a long, narrow, symmetrical spindle centred on the rhachis. This records absorption and excretion periods of practically equal duration, and variable extent of reaction in the barbs concerned from the rhachis outward corresponding to rise and fall of concentration at the reactive site. In other words, it records rising threshold of reaction from the bases of barbs apically. This conclusion is confirmed by the series of increasing doses which cause progressively wider marks until the margin of the feather is involved. With the larger doses the period of absorption is reduced relatively so that the greatest diameter of the mark lies far above its axial centre. The definitive pattern on the feather was studied in its development in the germ; the gradient of thresholds begins dorsally and rises to the ventral triangle.

Lillie & Juhn (1932) attempted to explain thresholds of reaction within the individual feather germ by the principle of relation between rate of growth and threshold previously demonstrated for feathers of different rates of axial growth (Juhn *et al.* 1931). Specifically, they attempted to show that there exists a rather steep gradient of growth rate in each barb, high at the apex and diminishing towards the base; or in the germ, high next the ventral triangle and diminishing towards the rhachis. Juhn & Fraps (1934*a*) adduced cogent reasons for believing that the growth gradient was originally represented as much too steep; and later (Fraps & Juhn, 1936*a*) emphasized the equality of the rate of *axial growth* of the barbs in the germ throughout their entire length. While admitting 'reaction differentials' along the collar as described by Lillie & Juhn, they no longer specifically accept the principle of even a slight differential of growth rate along the length of the barb. I myself have definitely accepted the principle of equal *axial* growth rates of rhachis and barbs (Lillie, 1940), but believe it possible that a more accurate study of the added tangential growth to which barbs, but not the rhachis, are subjected may yield evidence of a gradient in the growth rate of barbs. Such a differential would necessarily be small in terms of actual measurements, but it might be effective nevertheless. For the present the tempting theory of rate-threshold relations within the individual feather germ, similar to those between different feathers, must be regarded as not

proved. The principle of threshold differentials within the feather germ nevertheless remains secure, whatever its cause.

The form of threshold differential along the collar just described is directly related to definite patterns artificially produced, such as bars, spindles, inverted triangles, etc., and these may be repeated along the axis of the feather by interrupted injections, the spacing of which produces interesting variations; the normal feather pattern, which persists outside the areas of reaction, is thus broken up into a new pattern of a negative sort. All such patterns may be determined physiologically by a transverse gradient of threshold combined with uniform axial growth. They may have an axial or a marginal location determined either positively or negatively. They may also be confined to one side of the vane if the threshold is uniformly higher on one side of the germ than on the other (cf. Lillie & Juhn, 1932, Figs. 51, 52). The limits of pattern determination in accordance with this principle have certainly not yet been adequately determined.

III. SUMMARY

1. Feather papillae are regarded as individually persistent throughout life, from the time of their origin between the sixth and ninth days of embryonic life. In each feather tract the order of origin is significantly related to dynamic field properties of the tract, such as growth rates, degrees of asymmetry, etc.

2. Recent work on the anatomy and development of the rhachis with special reference to barb relations and the independent origin of the central rhachis is considered; also the development of the after-feather and of the pulp and its blood supply.

3. By plotting axial growth and isochrones on the vane of the feather it is possible to relate any definitive locus to its time and place of origin in the germ, and hence to the conditions of its determination.

4. The papillae of definitive feathers may be transplanted, or subdivided, or parts of papillae of different tracts may be recombined. The results of the operations show that papillae have a definitive bilateral organization, like amphibian eggs, for example; that they are tract-specific; and that a dorsal field, including the central rhachis, exerts a dominant influence on development. Various abnormalities of feather form produced may be interpreted. Twins and chimaerae may be produced. Abnormalities artificially produced persist through successive generations of feathers from the same follicle. The mesoderm of the papilla is regarded as the seat of the initial inductions.

5. The melanophores of feather germs are derived from the neural crest. When transplanted to foreign fowl hosts they determine the donor pattern in the host, as, for instance, barring in a white host. The genotypic composition of the donor melanophore is the controlling factor in both colour and pattern of the host feather in these experiments in all breeds of fowl examined.

6. The growing feather germ is a very sensitive indicator; reactions may be read by pattern modifications in the finished feather and referred to their locus of origin in the germ (cf. no. 3).

7. The thresholds of reaction of feathers to female hormone increase in proportion to rates of axial growth. This is very conspicuous with reference to the discontinuous rates of different tracts, and is also to be observed with reference to gradations of growth rate within a single tract.

8. Within the individual feather germ of breast and saddle tract, threshold of reaction to female hormone and thyroxin rises from dorsal to ventral loci of the collar. These transverse gradients produce transverse patterns on the vane of the feather varying in form according to rise and fall of hormone in the blood stream by absorption and excretion after injection: the principle of rate of reaction which is inversely proportional to threshold is also a factor in the determination of pattern.

9. The principles of physiology of development may be applied to some problems of physiological genetics, as, for instance, in the case of barred patterns.

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The functions of this fibrous layer have been studied recently in mammals, and there is little doubt that the results obtained are applicable to all bony animals. Warwick & Wiles (1934), by marking this layer with Indian ink, have shown that during growth it expands evenly over its whole extent, so that the muscles attached to its surface are expanded harmoniously and retain their proportions. Since the underlying bone is growing longitudinally only at its margins there is a movement of the periosteum over the bone, the deeper cellular layer acting as a soft bed which permits this periosteal slip. Thus the tube of fibrous periosteum forms an essential part of the epiphysial mechanism of growth, for if it were not present and the muscles were attached directly to the bony shaft, the attachments would be left behind by the new growth at the margin. Again the periosteum helps to attach the cartilaginous epiphysis to the bony shaft, a function made clear by the intensive study of the scapular cartilage of the horse (Leppert, 1933), and later extended to other forms (Schwenkenbecher, 1935). Thus when the periosteum is cut it becomes relatively easy to separate the cartilage from the bone.

The detailed arrangement of the periosteal and endochondral bone in the long bones of several bony fishes has been described by Wisniewski (1935) from a study of transverse sections, and this work, so far as the bony parts are concerned, confirms the arrangement of the trabeculae of endochondral bone given above. The mode of growth, in fact, with the zones of flattened and hypertrophied cells, the bays of erosion and the endochondral bone laid down on their walls, and the special arrangement of the fibrous layer of the periosteum, show that the mechanism of growth is fundamentally similar in fishes and in land animals, and it seems justifiable to claim that the relatively simple structures found in fishes have the essential properties of epiphyses. The absence of secondary centres of ossification or calcification, and of any orderly arrangement of the cells in the zones of flattened and hypertrophied cells are certainly primitive features not usually found in tetrapods, but do occur at some stage in the ontogenetic development of each individual.

The geological age of the epiphysial mechanism is uncertain. Endochondral bone is known already in cephalaspids (Stensiö, 1932), so that Gegenbaur's (1898) suggestion, that in the long bones of fishes the endochondral bone and marrow which replaced the cartilage were much later phylogenetic developments than the periosteal bone, cannot be accepted as necessarily true. Probably the mechanism is as old as the origin of the bony fishes themselves, which, since several distinct lines are already differentiated in Devonian times, must be placed before that period (Holmgren & Stensiö, 1936).

III. TEMPORARY CESSATION OF GROWTH IN BONY FISHES

Fig. 2 A shows the basal end of a radial bone of a salmon, *Salmo fario*, taken from a specimen on its way to the spawning ground. As is well known, this fish takes no food during this journey, so that growth is at a standstill. The zone of flattened cells is hardly marked off from the rest of the cartilaginous epiphysis, for the cells are no longer multiplying. The cartilage is for the most part shut off from the marrow cavity by a closing plate of endochondral bone (*c.p.e.b.*), though active erosion seems still to be proceeding in the peripheral parts of the cartilage near the periosteal shaft (*b.e.*). Absolute cessation of erosion by the formation of a complete closing plate has been described in other fishes (Haines, 1934), and a similar mechanism is found in rats associated with the winter cessation of growth (Erdheim, 1914) and with starvation (Harris, 1933). In all

long bones, but the epiphysial structures with the zones of undifferentiated, flattened and hypertrophied cells are well developed.

(3) *Chondrostei and Dipnoi*

In these two groups it is known that the ancestral forms had well-developed endochondral bone, while in the present-day types this has been reduced or lost (Watson & Gill, 1923; Watson, 1925). In *Acipenser ruthenus* the cartilage passes through the shaft without interruption and is only marked off from the epiphysial region by a rather wider spacing of its cells (Fig. 1 D, *co.c*). But in view of the fossil history and the known tendency in several groups to the loss of endochondral bone, it seems certain that the apparently simple epiphysial structure in these groups is secondary rather than primitive. A similar specialization in the Amphibia will be discussed later in more detail.

V. CARTILAGINOUS EPIPHYSES OF PRIMITIVE TETRAPODS

The early tetrapods appear like the fishes to have had cartilaginous epiphyses, for no secondary centres are known in fossil forms up to the end of Permian times. Among living groups which now possess cartilaginous epiphyses, the Chelonia, Crocodilia, urodele amphibians and birds, it is only the first two that appear to have retained the early type of structure, for the other groups are specialized in one feature or another. This structure can be directly compared with that in bony fishes.

The epiphysis has the usual arrangement of the cells (Wallis, 1927; Haines, 1938), but, particularly in the mature animal, the zone of undifferentiated cells is relatively reduced, so that the growth zone of flattened cells (Figs. 1 E, 3) comes to lie nearer the articular zone (*z.a.c.*) and to form a layer lying parallel to it. This gives a thinner and firmer epiphysis than is usually found in bones of similar size in fishes.

Again the individual cells of the growth zone are no longer scattered irregularly in the matrix, but are grouped more or less regularly in longitudinal columns (*c.clm.z.g.*). Dodds (1930) has made the important discovery that in mammals each group of flattened cells is the product of the repeated division of a single mother cell, which has divided a certain definite number of times, so that the number of the resulting daughter cells is a power of two. The columns are hardly visible in the young animal (Fig. 3), but become more pronounced with advancing age.

The regularity of the arrangement of the cells in the growth zone leads to a corresponding column formation in the zone of hypertrophy (*c.clm.z.h.*), whose matrix is calcified to within a short distance of the growth zone. So the marrow processes have an orderly arrangement imposed on them by the matrix which they are eroding, and come to form a more or less regular series of cavities which radiate towards the growth cartilage. The endochondral bone again, laid down on the sides and ends of these cavities, necessarily forms a series of trabeculae which show a simple arrangement with the longitudinal members radiating towards the growth cartilage.

It is clear then that the regularity of the arrangement of the endochondral bone is determined by that of the cells in the growth zone, and that the direction in which the trabeculae radiate is determined by the shape of the growth zone as a whole, and eventually by the arrangement of the articular surface which overlies the growth zone, and which the growth zone approaches during growth. So, though the actual cartilaginous epiphyses are not preserved in fossils, some evidence of their arrangement can be

gathered from the study of the endochondral bone, which is often very well preserved indeed (Hasse, 1878). An examination of fossil forms, particularly *Dicynodon* and an unknown amphibian of Rhaetic age in the British Museum, R. 299 (Haines, 1938), leaves no doubt that the Crocodilia and Chelonia preserve a primitive form of epiphysis which has remained unaltered throughout tetrapod development.

VI. LOSS OF ENDOCHONDRAL BONE IN URODELA

The urodele amphibians, so important in Eggeling's (1911) scheme of the evolution of epiphyses, are now known to be very highly specialized, and the direction of the evolutionary trends as understood by him must now be reversed. In caducibranchs such as *Salamandra* (Fig. 1 F), where the endochondral ossification has been described in detail by Klintz (1911) and *Molge* (Retterer, 1917), the general arrangement of cartilaginous epiphyses and the endochondral bone is similar to that in the primitive type just described,

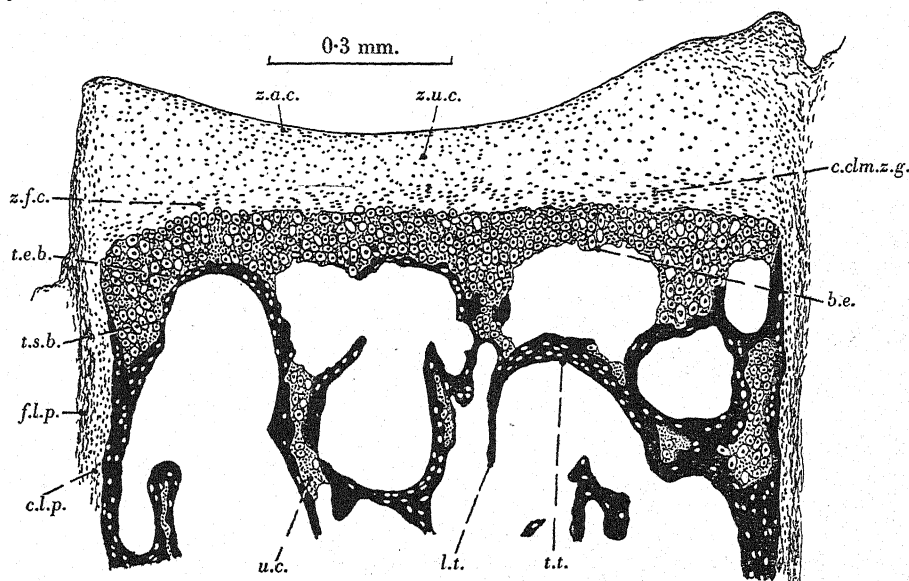


Fig. 3. Head of the radius of a young *Emys orbicularis*, illustrating the primitive type of structure in tetrapods. The cartilage columns (*c.clm.z.g.*) are not yet very distinct but can be made out here and there. The bays of erosion (*b.e.*) are wide, and the endochondral bone is laid down on their surfaces, giving a primitive type of trabecular structure.

though the zone of undifferentiated cells is rather large and the cell columns of the growth zone are poorly developed. The endochondral bone is well developed and regularly disposed.

In cryptobranchs and still more in perennibranchs (Fig. 1 G, H), on the other hand, all trace of any regular arrangement of the flattened cells has been lost, and the marrow, though it may erode the cartilage at several levels (*b.m.*), never completely breaks the continuity of the original embryonic rod. Endochondral bone (*e.b.*), where developed, lines the walls of the cavities in isolated patches, which since they often have no connexion with the periosteal bone of the shaft, can have little function. In *Proteus*, studied intensively by Eggeling (1911), it is only in the larger bones that there is any marrow at all.

Now Eggeling, following Gegenbaur (1898), though he knew of endochondral ossification in fishes, places the *Proteus* structure first in a morphological if not in a phylogenetic

series, and shows how the more complex types might have been derived from it, and this reasoning is again developed in his recent review (1938). But with our present knowledge of the structure of fishes and of primitive tetrapods, both living and fossil, we can understand that the urodele structure represents a peculiar sideline of evolution (Haines, 1938).

The reason for the loss of endochondral bone in Dipnoi, Chondrostei and Urodela is difficult to decide. The metabolic cost of maintaining a given volume of cartilage is very low as compared with other tissues, about one-tenth of that of connective tissue (Bauer, Ropes & Waire, 1940). Klintz (1911) found that in his laboratory specimens of *Salamandra* ossification was far in advance of that in wild specimens of similar age and size, probably on account of the more plentiful diet in the laboratory. Again cartilage is resilient, and may be well suited to resist the shocks encountered by a bottom-living animal moving about amongst stones. The groups that have reduced their endochondral bone agree in being such bottom-living forms with probably a rather low metabolic rate, and in having a pliable, tough bodily structure rather than the more rigid and precise form of a predatory fish or a crocodile. Even the selachians may have developed from more rigid ancestors, though their precise phylogeny is unknown (Moy-Thomas, 1939). The whole range of cartilaginous animals needs further biological investigation.

VII. MATCH-HEAD EPIPHYSES OF ANURA

The epiphyseal structure of the Anura is very difficult to review. Froböse (1927), in a paper which follows the lines laid down by Eggeling (1911) for urodeles, has given very detailed descriptions of a large number of specimens, finding a great variety of structures. In *Hyla brachiata*, for instance, he has described processes of the marrow that penetrate into the epiphyseal region, in *H. arborea* processes from the perichondrium which vascularize the epiphysis. But in the absence of any illustrations of these difficult points it is impossible to determine the nature of the structures he describes, whether for instance the perichondral processes are cartilage canals or marrow buds. Under the circumstances many of these peculiarities must be passed over until such time as some new worker undertakes the task of reinvestigating the problem.

Fortunately, the ordinary structure of anuran epiphyses is well known from the work of several authors, particularly Kastschenko (1881) and Lubosch (1927). The epiphysis is set on the shaft as the head of a match on its stalk, the cartilage of the epiphysis overhanging the shaft by a lappet formation (Fig. 1 I, J, *lap.*) so as to leave a relatively narrow groove filled with connective tissue between the epiphyseal lappet and the part of the shaft it surrounds (*c.t.l.s.*). This highly vascularized tissue acts as a cellular layer of the periosteum for the bone and as a perichondrium for the inner surface of the lappet. The fibrous layer of the periosteum is attached to the free margin of the lappet, and so does not pass into the groove. The lappets are not found in the young animal (Kastschenko, 1881, larval *Bufo viridis*; Haines, 1938, *Xenopus laevis*), and are not developed in all bones, being absent, for instance, at the lower end of the humerus.

Within the epiphysis is a large mass of calcified cartilage which extends into the lappets, forming a peculiar type of epiphyseal centre (*2ry.c.cal.*). Thus the growth zone, which remains hyaline, comes to lie between two heavily calcified regions, the secondary centre of the epiphysis and the calcified region of the hypertrophied cells. The general pattern of the calcification is very well illustrated by Lubosch (1927) for several species.

In older animals the interior of the epiphysis may be ossified in continuity with the shaft and the growth zone entirely destroyed, but the details of this epiphysial invasion are unknown.

Endochondral bone may be well developed as in *Rana esculenta* (Kastschenko, 1881), or in other, doubtless more specialized, forms may be altogether absent as in *R. temporaria* (Haines, 1938). The match-head type of epiphysis, which provides a very firm attachment for the cartilage to the end of the tubular shaft, has rendered the development of endochondral bone unnecessary. The epiphyses as a whole give the impression of a recent and peculiar specialization for jumping imposed on an earlier structure such as is found in the less specialized urodeles, for example *Salamandra*, for these animals share with the anurans the lack of regularity of the cells in the growth zone.

VIII. SECONDARY CENTRES OF *SPHENODON*

The most primitive living tetrapod possessing secondary centres is undoubtedly *Sphenodon* (Haines, 1939), in which the first reptilian secondary centres to be described were recorded in the spines of the vertebrae by Albrecht (1883) and of which I have examined several developmental stages. The antiquity of these centres is attested by their

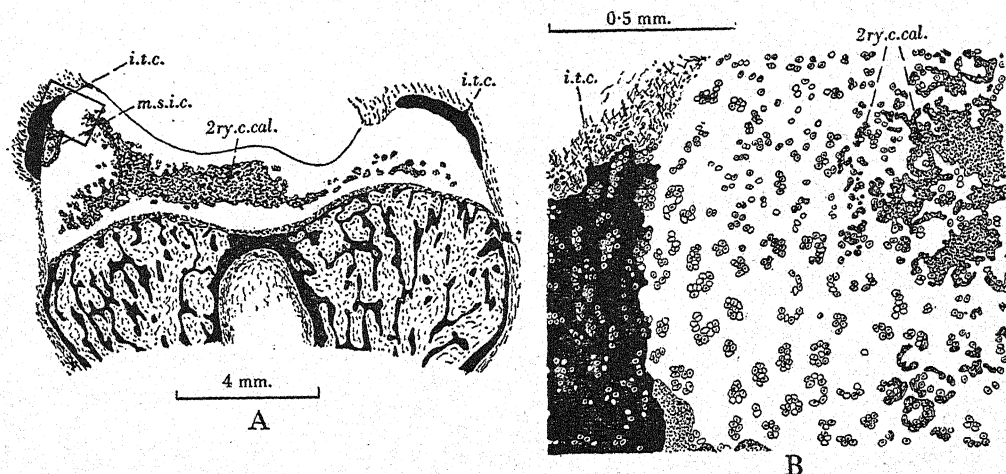


Fig. 4. A, lower end of the humerus of *Sphenodon punctatum*, the most primitive living animal possessing secondary centres. The main centre is calcified (2ry.c.cal.), and two intratendinous centres (i.t.c.) form the epicondyles. B, enlargement of the part outlined in A.

presence in the Jurassic *Sapheosaurus*, the earliest known animal to possess such centres (Fuchs, 1908). In the young animal, where the secondary centres are not yet developed, the zone of undifferentiated cells is relatively large, but otherwise the structure resembles that in *Chelonia*. The secondary centre develops as a large mass of heavily calcified tissue which spreads irregularly into the cartilage matrix (Figs. 4, 5 A, 2ry.c.cal.). In its full development the epiphysial centre occupies the greater part of the cartilage, but leaves an uncalcified region peripherally next the articular surface and the perichondrium.

In the adult animal the tissues of the shaft have grown through the growth cartilage which is entirely destroyed, and have replaced the calcified region by endochondral bone and marrow. No specimen is actually known showing this process, so that the piercing of the growth cartilage in this animal remains an inference from the adult structure rather than an observation.

epiphyses resemble in many ways those of lizards so that here again a *Sphenodon*-like phase may have been passed through in some ancestral form.

IX. FUNCTIONS OF EPIPHYSES AND OF SECONDARY CENTRES

It has always been understood that epiphyses in general are a compromise arrangement to allow for the simultaneous growth and function of bones. But attention has usually been directed to the secondary centres in the epiphyses rather than to the cartilages themselves, and indeed in human anatomy the word epiphysis is usually taken to mean the secondary centre which forms so conspicuous a feature. Now, however, that the cartilaginous epiphyses of fishes and of the more primitive tetrapods are well understood, and are known to be more primitive than epiphyses containing secondary centres, it may be well to discuss the functions of such primitive structures, and then to consider those of the centres found in them.

The epiphysial mechanism has been developed because bone is one of the tissues that can grow only by accretion, and not by interstitial expansion. Where bone is covered by connective tissue, as on the surface of the shaft, a periosteum can be developed, whose inner layer can increase the size of the bone. In the articular region the actual surface is made of cartilage, so that the longitudinal growth of the bone involves replacement of this tissue. The existence of a large mass of cartilage rather than a thin covering is probably associated chiefly with the firm attachment of the fibrous layer of the periosteum, so that the epiphysis takes the pull not only of the muscles directly inserted into it but also of those usually described as being inserted into the shaft of the bone. Thus even in the most primitive type of cartilaginous epiphysis the skeletal part is essentially made up of two cartilaginous masses attached to each other by a fibrous tube, with the shaft of the bone acting as a strut to hold them apart, and with special growth cartilages to add to the length of the bone.

It may be of interest to notice that whereas in most long bones of the branchial or appendicular skeleton there is but one centre of ossification, with growth zones developed in the cartilage on either side of it; in other regions of the skeleton there may be several primary centres, each with an associated growth zone developed opposite the growing margin of the periosteal bone. Thus in the Meckel's cartilage of teleosts, studied intensively by Haines (1937 *b*), this has led to a peculiar reversed form of the epiphysial mechanism, with the cartilage ossified at either end and the growth zones adjacent in the centre of the cartilage. The actual appearance of the growth zones is similar to that found in the epiphysial mechanism, so that the development of a plate of flattened cells seems to be conditioned by the advance of the periosteal bone, and is not related especially to the epiphysis itself. A somewhat similar arrangement is found in the limb girdles of mammals, which are ossified from several centres, leaving a thin growth cartilage in the region where these centres meet.

It has been seen (§ V) that the longitudinal trabeculae of endochondral bone necessarily radiate towards the growth cartilage, and that where the growth cartilage comes to lie in the mature animal near the articular surface, the trabeculae radiate towards that surface. This gives a reasonably satisfactory mechanical arrangement of the bone, particularly when the articular surface is set directly on the end of the shaft, but when the articular surface is offset from the main axis of the shaft it is not quite so satisfactory. If, however, the articular cartilage and the growth cartilage can be separated, then each

can be arranged in the most advantageous position, the one for giving a good joint surface, and the other for directing the arrangement of the trabeculae. The disadvantage of this separation would be, in a land-living animal, the weakness of the large mass of undifferentiated cartilage between the two layers, but this weakness can be overcome by the introduction of the secondary centre of calcification or ossification in place of the cartilage.

Now Parsons (1905) divided secondary centres into three groups, pressure, tension and atavistic epiphyses, the centres under discussion belonging to his first group. Certainly these centres are developed in the cartilages near joints, and are subject to direct and oblique pressures, but the actual development of secondary centres seems to be associated particularly with the arrangement of the endochondral bone (Haines, 1938). The hormonal or other mechanisms involved will of course affect all the cartilages of the requisite size and shape in the body, so that it is probably wrong to demand a definite function for each individual centre. Parsons's tension epiphyses are probably equivalent to my intratendinous centres (§ XVII), while the existence of his atavistic group is very doubtful.

There have been numerous evolutionary changes in the epiphysial centres, and these will be discussed later. But these changes have been concerned with the strengthening and the better nutrition of the centres themselves and do not affect the conceptions here put forward concerning the fundamental use of the centres to the animal.

X. BONY SECONDARY CENTRES OF LACERTILIA

In the Lacertilia the bony secondary centres were first discovered and listed by Dollo (1884). Their naked eye appearance and general arrangement have been described by Moodie (1908) and Fuchs (1908), and their histological structure by Heidsieck (1928) and Haines (1941). Thus knowledge of their distribution and structure presents a pleasing contrast to that of most other submammalian groups.

The secondary centre when it first appears consists of a diffuse calcification of the matrix in the interior of the cartilaginous epiphysis (Fig. 6, *zry.c.cal.*). This is later eroded by one or more marrow processes (*m.p.zry.c.*), which grow in from the perichondral tissues (*p.c.t.*) and which expand to form a series of cavities. The active erosion of the calcified matrix is indicated not only by the bare surface of the calcified cartilage, but also by the presence of numerous osteoclasts (*o.cl.*) in the processes. At a later stage (Figs. 5 B, 7) endochondral bone (*e.b.zry.c.*) is laid down in the walls of these cavities forming a typical bony centre which adapts itself to the shape of the cartilaginous epiphysis, but in most bones does not reach the surface, so that a thin layer of cartilage surrounds it on all sides. The spread of the bone is preceded by the spread of the calcification in the matrix (*cal.m.zry.c.*), which becomes denser as growth proceeds, so that the bone is everywhere separated from the hyaline cartilage by this calcified tissue.

In the mature animal growth is terminated by the exhaustion of the supply of young cells in the growth cartilage. All these cells ultimately become hypertrophied, and this is followed by calcification of the matrix between the epiphysial centre and the shaft, at first extending over limited areas only, the stage of co-calcification (Haines, 1941). Later the marrow of the shaft grows through these co-calcified areas, so as to join that of the secondary centre. The remains of the growth cartilage are now gradually removed, but in some forms may persist indefinitely as walled-off masses of calcified cartilage

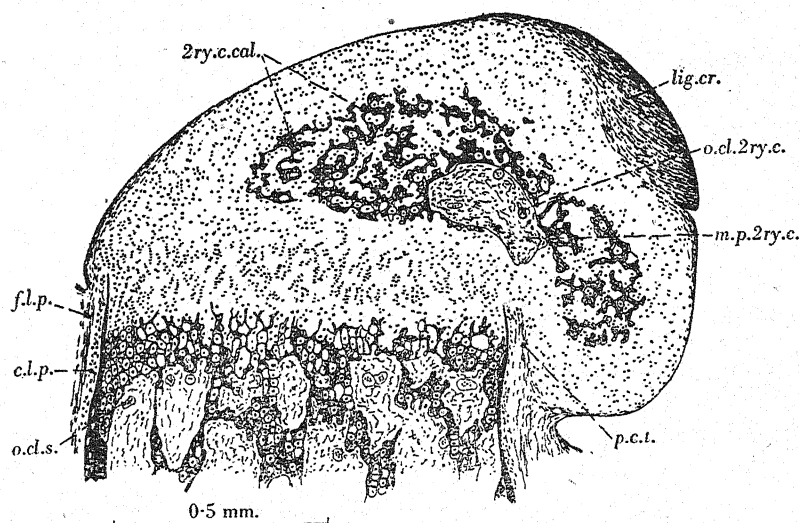


Fig. 6. Lower end of the femur in a young *Agama kirkii*, showing the diffuse calcification of the secondary centre, and an early stage of erosion by osteoclasts (*o.cl.2ry.c.*). Endochondral bone has not yet appeared.

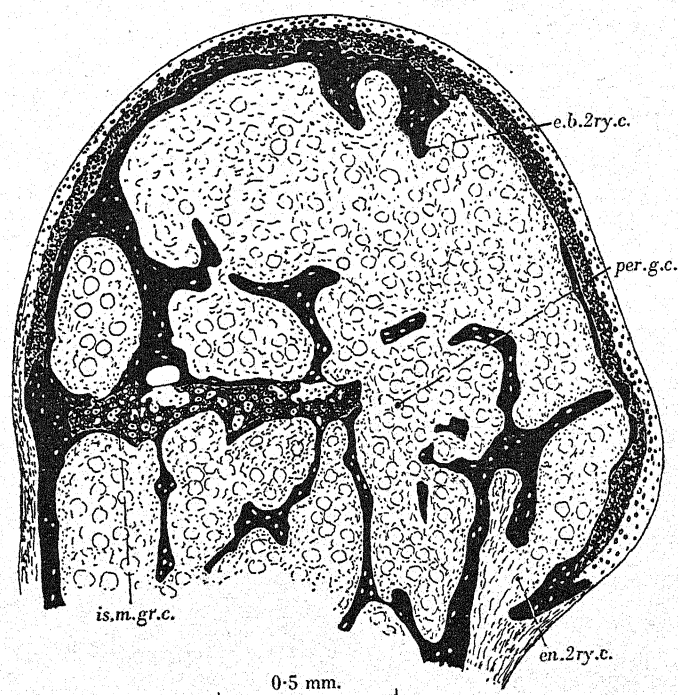


Fig. 7. Lower end of the femur of an adult *Zonurus cataphractus*. The original region of entry of the tissues forming the secondary bony centre can still be seen (*en.2ry.c.*). This centre has expanded so that the cartilage is now reduced to a relatively thin articular layer and to a growth cartilage, of which all the cells are now calcified and hypertrophied (*is.m.gr.c.*). The growth cartilage is perforated by the marrow of the shaft, which is continuous with that of the secondary centre.

(Fig. 7, *is.m.gr.c.*). Probably the processes of union are similar in mammals, but they have not yet been studied in sufficient detail for exact comparison.

The great majority of lizards appear to conform to the type described. In *Lacerta* (Vialleton, 1924; Wallis, 1928; Nauck, 1936, 1938; Haines, 1940) the cells of the calcified secondary centre have, before ossification sets in, a peculiar radiate arrangement. In *Phyllodactylus porphyreus*, and probably in other very small lizards, the secondary centre, though well developed as a calcified area (Fig. 5 C, *2ry.c.cal.*), is never ossified, but this is clearly a peculiar specialization. Only the Varanidae, discussed later, offer any marked differences.

XI. ORIGIN OF SECONDARY CENTRES

In turtles, and presumably in all primitive tetrapods, the long and short bones ossify by rather different mechanisms (Haines, 1938). In the long bones the primary centre appears by the formation of a periosteal cylinder surrounding the cartilaginous rod, and the erosion early involves the whole thickness of the shaft of the rod. So the cartilage is at an early stage cut completely into two separate parts, and these eventually give rise to the two epiphyses. In the short bones, on the other hand, the calcification is at first confined to the interior of the cartilage, and is separated everywhere from the surface by a continuous layer of hyaline matrix. Eventually the centre may spread so as to reach the surface of the cartilage, but this is at a relatively late phase of growth, and until it reaches the surface there is no periosteal bone, though endochondral bone may be well developed in the interior of the cartilage. Now these differences were presumably well developed in the early tetrapods of, say, Carboniferous times, but when, much later, ossified epiphyses appeared, the mechanism of ossification was by the methods already developed for short bones. Possibly the enlargement of the cartilaginous mass of the epiphysis in the embryo automatically sets in train the hormonal or other mechanisms involved in this type of ossification.

The secondary centres of ossification, once they are developed, and the centres of the short bones resemble each other very closely in their further evolution. Such features as cartilage canals, for instance, if they are developed in the one region, will also be found in the other.

XII. CARTILAGE CANALS OF *VARANUS*

The genus *Varanus* contains the only living representatives of a very distinct line of lizards marked off from other groups by many characteristics. The line may have been more diversified in the past, for some of the Mesozoic aquatic reptiles are probably derived from it. Parsons (1905), Moodie (1908) and Fuchs (1908) have figured gross preparations showing the form of the secondary centres. Lubosch (1910) has given good figures of microscopic preparations of what is obviously the epiphysial region of a member of this genus, but since he mistook the animal he described for *Sphenodon*, to which it bears little structural resemblance, and since he misunderstood the nature of the tissues he figured, this work can be passed over.

As in other lizards the secondary centres are developed in the newly hatched animal as diffuse calcifications of the matrix (Haines, 1941), but are distinguished by the possession of a remarkably well-developed system of cartilage canals (Figs. 5 D, 8, *c.can.*). These form tree-like systems of connective tissue which enter the cartilage from its

perichondrium-covered surfaces, and end as a series of blunt-ended branches in the matrix, which is hyaline in the region directly surrounding each branch. In the interior of each large canal, embedded in the connective tissue, is a central artery and a peripheral plexus of veins, while in the small canals there is only a capillary plexus. The whole system forms a means of carrying a blood supply into the interior of the cartilage, so that the cartilaginous epiphysis can grow to a large size before it need be replaced by a second-

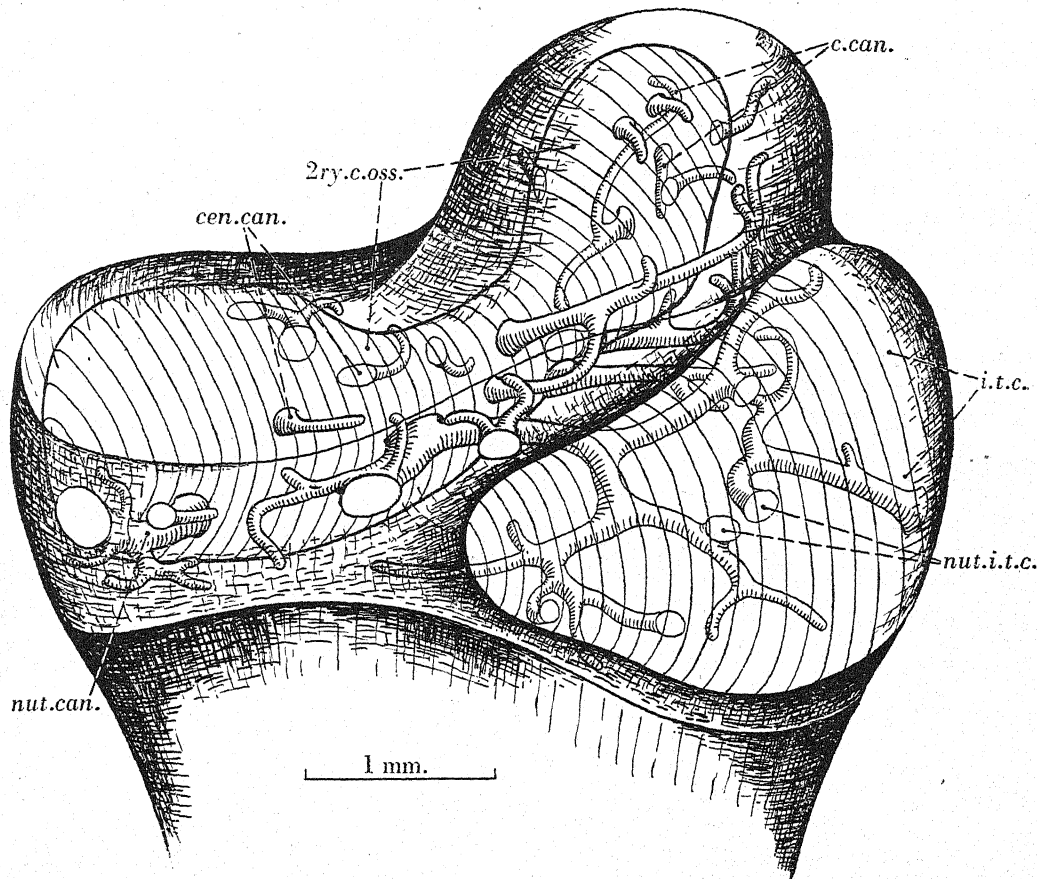


Fig. 8. Celluloid reconstruction of the lower end of the radius of *Varanus exanthematicus*. In the cartilage is seen the main epiphysal centre (2ry.c.oss.), and on its surface an intratendinous centre (i.t.c.) which is now invading the cartilage. Cartilage canals (c.can.) are well developed, and many of them supply the bone (nut.can. and nut.i.t.c.).

dary centre. It is noteworthy that the varanids include the largest of the lizards both living and fossil.

In mammals the nature and growth of cartilage canals have been considered particularly by Hintzsche (1928, 1931), Hurrell (1934) and Haines (1933, 1937). Hintzsche believes that the canals are formed by an actual invasion of the cartilage by the connective tissues of the perichondrium, and gives some evidence which might indicate that solution of the matrix takes place. Haines believes rather that the perichondral tissues become included in the cartilage by the growth of the matrix around them, and that there is no destruction of matrix. Both authors are agreed that the primary function is one of nutrition and that the canals are only secondarily involved in the processes of

ossification. Another accessory function is the carriage into the interior of the cartilage of a supply of young undifferentiated cells, which, by the development of matrix, add to the cartilage substance. But the importance of this injection of new cartilage into the interior of the old model cannot be determined.

When ossification does set in (*2ry.c.oss.*) the tissues which form it are derived from the cartilage canals which already occupy the interior of the cartilage, and not as in other lizards directly from the perichondrium. The canals which now lead to the bony centre form its nutrient canals (*nut.can.*), and as the centre grows these nutrient canals are reduced to short stumps. Again, some of the branches become separated from their parent trunks by the growth of the centre, and these now form the centrifugal canals (*cen.can.*), which run from the bony centres to end blindly in the cartilage. Eventually the bone spreads so as to obliterate all trace of the canals, leaving only the thin layers of articular and growth cartilage which persist throughout the life of the animal. At this stage it would be impossible to decide by examination whether canals had ever been developed in the epiphysis.

XIII. BONY CENTRES AND FINE ENDOCHONDRAL BONE OF MARSUPIALS

In marsupials (Haines, 1941) the structure of the epiphyses is relatively simple. There are no cartilage canals, and the secondary centre appears as a sharply limited area of calcification, a contrast to the large diffuse area found in typical lizards, which the marsupials otherwise resemble. This calcified centre is soon ossified by a perichondral ingrowth which forms endochondral bone and marrow (Fig. 5 F).

The endochondral bone of most mammals is much finer in structure than that of reptiles (Retterer, 1917; Lubosch, 1924). In reptiles each marrow process corresponds in width to several rows of cartilage cells, and the spaces between the marrow processes are often correspondingly wide, and contain both matrix and cartilage cells (Fig. 5 B, *u.c.*). In mammals, on the other hand, the processes are so fine that each erodes a single row of cartilage cells at a time, and they are set so closely together that in rapidly growing long bones there is a separate marrow process developed for each row, and no cartilage cells are left intact. In the slower-growing bones, on the other hand, erosion of the cartilage may be less complete. Dodds (1930) found that in the region of hypertrophy it was only the cartilage matrix forming the thicker walls between adjacent rows of cells that was calcified, while the transverse septa that separated the individual cells of a single row from one another remained uncalcified. This may account for the presence of osteoclasts near the ends of the marrow processes in reptiles (Fig. 6, *o.c.l.s.*), where the marrow processes are thick and erosion involves both the longitudinal walls and the transverse septa, while in mammals they are not found in this region, for the destruction of uncalcified cartilage matrix can be carried out by the other cells of the bone marrow.

So the endochondral bone when first laid down consists of a series of fine tubules each lining the walls of a space once occupied by a single row of hypertrophied cartilage cells, instead of both the walls and ends of the much larger bays of erosion found in reptiles. Lubosch (1924), who has studied endochondral ossification in detail, has distinguished the two modes of growth as the intramedullary type of ossification in reptiles and the intracartilaginous type in mammals. There is, however, a rather gradual transition from one type to the other, some lizards, for instance, showing almost as fine a structure as the typical mammals.

In mammals the three-layered sheets, each formed of a core of uneroded calcified cartilage with its two coverings of endochondral bone, are known as the primary trabeculae (Fig. 5 F, G, 1ry.t.), and their arrangement has been studied by many authors, particularly Lesser (1888) and Bidder (1906). They found a regular series of bony lamellae all arranged longitudinally, and connected with one another at their edges as the walls of a honeycomb. Harris (1933) described a persistence of some of the transverse walls between the rows of cartilage cells as a scaffolding for transverse trabeculae, but this does not agree with Dodds's (1930) observations, and cannot be confirmed by a study of transverse sections through this region, in which such trabeculae, if they existed, should be particularly obvious.

Soon after their formation the primary trabeculae are replaced by a series of secondary trabeculae (2ry.t.), distinguished by the absence of any core of calcified cartilage. The fate of the calcified cores is still very doubtful. Certainly most of the primary trabeculae are destroyed altogether, and osteoclastic activity is always very conspicuous in a zone a little distant from the advancing face of the marrow. Schaffer (1888) was of the opinion that all the primary trabeculae were destroyed and that the secondary trabeculae were entirely new formations. Ziba (1911), Mjassojedoff (1922) and others have revived the older concept of a direct metaplasia of cartilage into bone, by uniform calcification of the ground substance and by a change of the cartilage cells into osteocytes, so that some at least of the primary trabeculae may be preserved in the secondary system. A similar metamorphosis of cartilage into bone has often been described in amphibians and reptiles as 'sclerosis of cartilage' (Eggeling, 1911; Froböse, 1927; Heidsieck, 1928). A third alternative, that the endochondral bone gradually spreads into the cartilage by a process of 'creeping replacement' at the cartilage-bone interface, first suggested by Marchand, (1901) is supported by Ham (1932).

The secondary trabeculae, which seem to bear some relation to the function the bone will be called on to perform, are peculiar to mammals, for in reptiles, though the bone as first laid down may be destroyed or thickened, its fundamental pattern is never altered. Unfortunately, though a vast literature has accumulated dealing with the anatomical pattern of this trabecular system, following the work of Wolff (1892) and others on its mechanical arrangement, the details of its development have never been studied.

XIV. CARTILAGE CANALS AND SECONDARY CENTRES OF EUTHERIAN MAMMALS

The canals of eutherian mammals (Hintzsche, 1928, 1931; Hintzsche & Schmid, 1933; Hurrell, 1934; and Haines, 1933, 1937) are similar to those described in varanid lizards, though they are rather more numerous, and each individual canal is less branched (Fig. 5 G). As in varanids, when the ossification centre develops, the canals provide the tissues which form it, and supply it with blood vessels (*nut.can.*), and the ends of the canals may be severed from their parent trunks (*cen.can.*). But in mammals at certain stages of their development some of the branches of the canals turn down into the growth cartilage, running between the columns of flattened cells, and may perforate the growth cartilage so as to reach into the hypertrophied cartilage (*per.c.*). Some of the canals pass even farther so as to reach the marrow cavity and the shaft, in which case a direct communication of the blood vessels from the perichondrium is established with those of the shaft.

Probably the growth of these perforating canals is passive. The cells of the growth zone are continually being recruited from the undifferentiated cells of the cartilaginous

epiphysis, so that, as the products of the division of these cells are continually passing off as hypertrophied cells, the cells constituting the growth zone are not the same individuals throughout the period of growth, and the growth zone itself moves slowly through the cartilage. In this process some of the ends of the cartilage canals may be caught up in the growth zone, and with the elongation of the cell columns may be passively carried through to the tissues of the shaft. Hintzsche (1928) believed rather that the marrow of the shaft took an active part in the formation of these canals, but the evidence offered in support of his contention, a specimen showing a marrow process longer than its neighbours projecting towards a cartilage canal, is far from convincing. Obliteration of the perforating canals is a constant phenomenon at a slightly later stage of growth, and it seems most likely that in Hintzsche's specimen such an obliteration had occurred, leaving the lower end of the canal as a projection of the marrow.

When the bony centre develops, the perforating canals may persist as communicating canals (*com.c.*) carrying vessels between the shaft and the epiphysal centre, and endochondral ossification may spread up the wall of these canals (*e.b.can.*) towards the growth cartilage. It is such canals, usually very conspicuous at the time of ossification of the secondary centre and shortly after, that have given rise to the current description of marrow buds which perforate the growth cartilage from below, eroding their way into the cartilaginous epiphysis, so as to set up there independent ossification centres, the 'canales vasculosi ossificantes' of Bidder (1906). A study of the canal system as a whole by serial section and reconstruction, however, leaves no doubt as to their true nature. How important they may be in carrying vascular supply to the secondary centre is unknown, but certainly Harris (1929) goes too far in denying their existence altogether. In the early stages of ossification they are usually very conspicuous, and it is easy to understand that the sudden demands of a new ossification centre might well be more than the original canal system could easily supply, and that the blood flow in the perforating canals might be reversed to make good the deficiency; but the direction of the flow has not yet been demonstrated. In later stages of growth, as in Harris's specimen, these communications with the shaft are obliterated.

Since cartilage canals are absent in marsupials but developed in eutherian mammals, they seem to have been evolved since the origin of the mammals themselves. But some small mammals such as the rat and mouse (Fig. 5 H) develop their bony centres as do marsupials, directly from the perichondrium (Haines, 1933). Probably the ancestors of these animals had canals, for they are well developed in other rodents, but they have been lost as the cartilages up to the time of ossification are so small that they do not need a special mechanism for their nutrition. In the same animals Dawson (1929, 1934, 1935) has found that several of the epiphysal centres fail to unite with the shaft, and remain separate throughout life, and he has investigated the histology of these structures.

In animals with cartilaginous epiphyses these grow, as do cartilages in general (Retterer, 1900), partly by interstitial expansion and partly by the formation of new cartilage in the perichondrium and subperichondral region. In mammals the distribution of mitotic and amitotic cell divisions (Harris & Russell, 1933; Elliott, 1936) indicates an active growth of the cartilage in a zone a little below the perichondral and articular surfaces, and this zone is peculiarly liable to pathological changes in response to pituitary extracts (Silberberg, 1936). Some of the growth below the articular surface, however, may be for the purpose of making good the cartilage worn off from the surface during movement (Meyer, 1924). The bone, when it is developed, at first spreads evenly into

the surrounding cartilage, but in the pig, studied by madder feeding by Payton (1933) and histologically by Krompecher (1937), there is some resorption of the bone on the surface facing the growth cartilage. How general this may be is unknown, but it has not so far been observed in other species.

XV. PECULIARITIES OF MONOTREME CANALS

The monotremes and birds are discussed after eutherian mammals rather than in their more usual zoological position, as their structure must be interpreted in the light of what is known in species more thoroughly understood. Unfortunately only one indi-

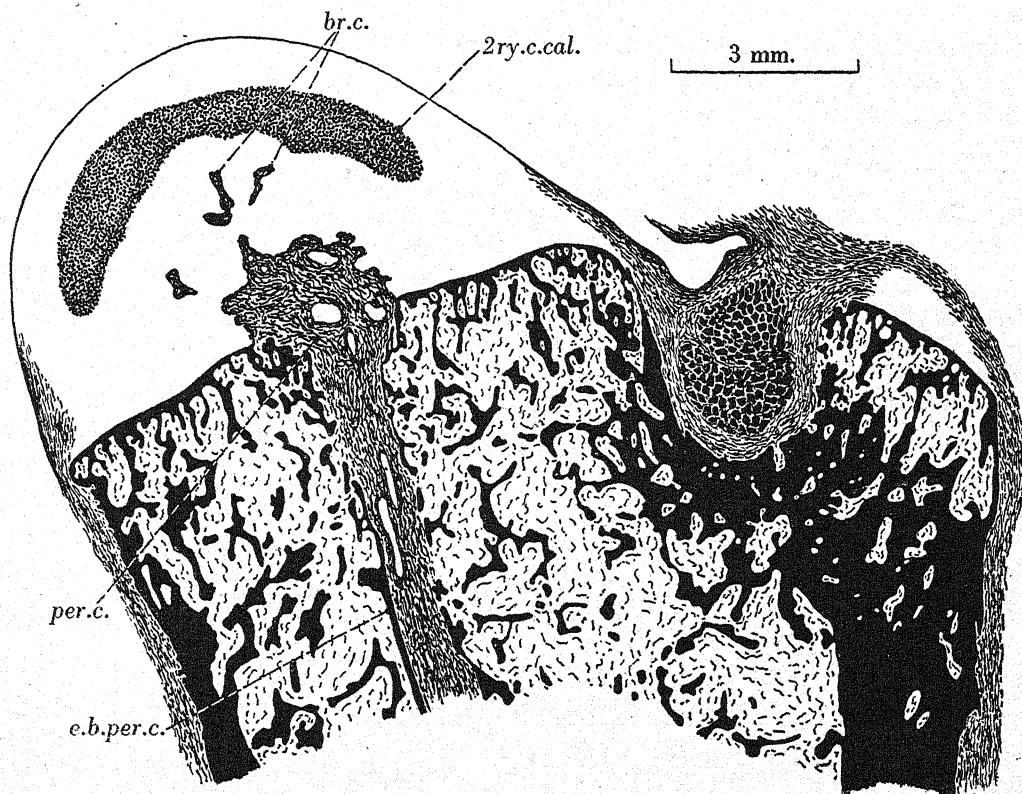


Fig. 9. Upper end of the femur of *Echidna* sp., showing a large secondary centre of calcified cartilage (2ry.c.cal.), and a system of branches (br.c.) arising from a large canal which enters the cartilage from the shaft.

vidual belonging to the monotremes has been examined for epiphysial structure, and Fig. 9 is the only extant picture. But the conditions found are so unlike those in any other group that they seem worth discussion. In each condyle of the lower end of the femur and in its head is a large calcified mass (Figs. 5 I, 9, 2ry.c.cal.) which follows the shape of the cartilaginous epiphysis, being convex on the side towards the articular surface and concave where it faces the growth cartilage. Passing through the growth cartilage is an enormous perforating canal (per.c.), which branches in the matrix (br.c.). In the condyles of the femur, where ossification of the secondary centre has already begun, the tissues of the centre are derived from this perforating canal, which has eroded

the undersurface of the calcified mass and has given rise to several points of ossification, which would shortly, no doubt, have coalesced to form a single centre (*2ry.c.oss.*).

Apart from this single large perforating canal and its branches there are no canals whatever in the cartilage. On the other hand, the perforating canal is so large that even when the epiphysal cartilage is lost by maceration its course in the shaft can be seen in the dry bone, for it is walled off from the marrow spaces by a distinct sheet of bone (*e.b.per.c.*), and the depth to which this bony track can be followed in the shaft indicates that the canal has been developed for a considerable period of time.

Possibly this canal was developed as are the perforating canals of eutherian mammals, for it greatly resembles a canal figured by Bidder (1906) in the upper end of the tibia of a rabbit. If an ordinary canal system were at one time present in the monotreme, and if a down-turned end became continuous with the marrow of the shaft, then if the original system were to disappear, the end, having established a vascular connexion with the shaft, might persist as an independent structure. Clearly the whole question should be studied afresh by some worker who can get the necessary growth stages.

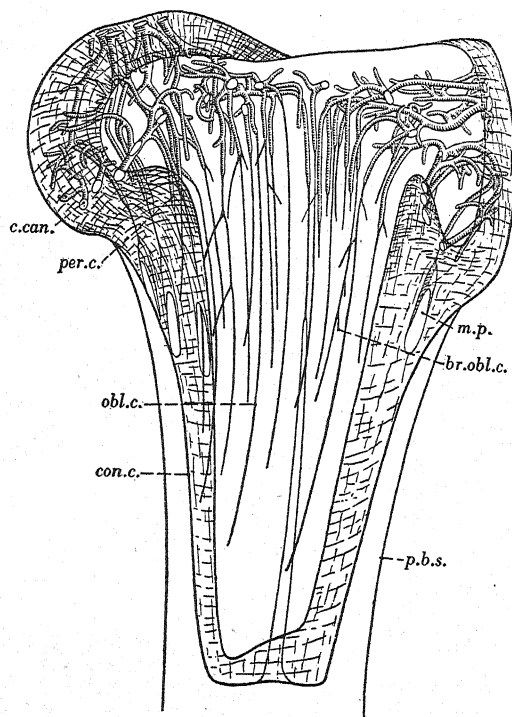


Fig. 10. Celluloid reconstruction of a thick section of the cartilage in the upper end of the tibia of *Gallus domesticus* about 10 days after hatching. The cartilage forms a long peg-like cone (*con.c.*), which fixes the epiphysis in the bony shaft. The canals ramify in the cartilaginous epiphysis, and their ends pass down into the cone, but soon become obliterated (*obl.c.*).

XVI. CARTILAGE CANALS AND PNEUMATIZATION IN BIRDS

Information about birds is quite extensive, but is scattered. Schöney (1876), Van der Stricht (1890), Brachet (1893) and Lubosch (1924) have given good diagrams showing the early spread of endochondral ossification, and the histological changes that occur in birds are perhaps better known than those of any other group, thanks to the work of Fell (1925).

Resorption of the hypertrophied cartilage is at first uneven, the marrow processes spreading up between the cartilage core and the inner surface of the shaft as so to isolate a large mass of cartilage which persists to a later date. At this stage then a long cone of this hypertrophied cartilage (Fig. 10, *con.c.*) projects from the cartilaginous epiphysis far into the shaft, forming the 'cône cartilagineux médullaire' of Van der Stricht, acting probably as a peg to hold the epiphysis in place until the endochondral bone is sufficiently strong to support the epiphysis without its help. A somewhat similar mechanism has been found by Haines (1938) in a young turtle, though there the marrow processes which isolated the cartilage were derived directly from new periosteal buds which had pierced

the bony cylinder to reach the cartilage. Again, from the existence of an isolated mass of hypertrophied cartilage in a young crocodile there was some evidence that at an earlier stage the cartilaginous peg had been developed, though this had never actually been seen. So it is reasonable to suppose that the peg mechanism is primitive for tetrapods and has persisted in birds. In later stages the peg is entirely resorbed.

Cartilage canals are well developed (Figs. 5 E, 10), but as they have not been studied in birds since the fundamental work of Hintzsche (1928) and others in mammals, they have been described as marrow cavities (Van der Stricht, *Meleagris*; Lubosch & Fell, *Gallus*). They enter as usual from the perichondrium, and as in mammals some of their branches turn downwards through the growth zone as perforating canals (*per.c.*). The lower ends of these branches may open into the marrow cavities of the shaft (Vialleton, 1919), and the marrow processes are guided in their growth by these canals and widen them from below, laying down endochondral bone on the walls of the widened parts (*e.b.can.*). This may give the impression in isolated sections that the canals are formed from the marrow processes, and Lubosch speaks of the whole canal system as a series of marrow processes which reach up into the cartilage to within a short distance of the articular surface. Fell, on the other hand, describes the canals on the two sides of the growth cartilage as separate structures, the marrow cavities of the epiphysis and shaft. The reconstruction shown in Fig. 10, however, the first to be published for birds, makes it quite clear that though the canals are well developed in birds they are essentially similar to those in mammals.

In later stages as in mammals the continuity between the canals and the marrow of the shaft is lost, though at the stage shown in Fig. 9 traces of the obliterated canals (*obl.c.*) are still well developed, and can often be followed to the ends of the marrow processes (*m.p.*). Some of these scars are branched (*br.obl.c.*), the branches ending blindly in the matrix.

In later stages still the cartilage of the epiphysis diminishes in size and the canals dwindle away, till when the cartilage is reduced to a relatively narrow articular layer no canals are left, a stage illustrated by Whiston (1940).

The only secondary centre usually recognized in birds is at the upper end of the tibia, where it has been noted by many authors, particularly Shufeldt (1886), Parsons (1905) and Fuchs (1908), but its minute structure has not been studied. On the other hand, Landauer (1931) has been able to demonstrate a great number of centres in his experimentally produced chondrodystrophic fowls, so that the mechanism for the development of these centres must be present in birds, though usually it remains latent. Possibly the ancestors of the birds may have had secondary centres at some stage of their evolution, and have lost them secondarily. The interior of the shaft is invaded by an air-sac system which grows out from the lungs, whose development has been studied by Blumstein-Judina (1905). The loss of secondary centres may be an adaption to this development, allowing the growth cartilage to approach the articular cartilage so as to make room for the expanding air sacs.

On the other hand, Latimer (1927), though he knew of the general opinion regarding the absence of secondary centres in birds, stated most emphatically that he had found epiphysal centres in all the long bones he used for his osteometric work, described how he had held them in place while he measured the bones, and stated further that they finally became ankylosed with the shaft. But Whiston (1940), incidentally to his work on experimental dislocation of the hip, has given excellent photographs of the normal

femur at several stages, and these show no secondary centres. It is perhaps possible that what Latimer described was the cone of cartilage with some endochondral bone attached to it, and that this became loose in the end of the shaft in the course of the preparation of his bones. A similar misinterpretation of an endochondral mass belonging to the shaft as an epiphysis has been corrected by Moodie (1908) in plesiosaurs.

XVII. INTRATENDINOUS CENTRES

Besides the typical secondary centres of ossification already described, there are often present in addition intratendinous centres, which begin as direct ossifications of tendons where these are inserted into the cartilaginous epiphyses. They were first defined as 'apophysial' as opposed to 'epiphysial' centres, by Bidder (1906), who studied the ossification of the tuberosity of the tibia. The centres begin by a simple calcification of the tendon fibres and ground substance, the tendon cells becoming enclosed in the calcified area as bone cells, but preserving their arrangement in rows or groups, whichever arrangement they had before the calcification reached them. Later the calcification spreads into the cartilage, and both calcified tendon and cartilage are for the most part eroded away and replaced by endochondral bone and marrow (Fig. 8, *i.t.c.*). In the later stages of development it may be difficult to determine whether any particular bony centre is an ordinary epiphysial centre which has spread outwards so as to involve the tissues on the surface of the cartilage, or an intratendinous centre which has spread inwards into the cartilage, but in origin these two types of centre are quite distinct (Haines, 1940).

The distribution of these centres is poorly known, for no survey of the structure of all the epiphyses in any one animal has ever been carried out. They occur already in *Sphenodon* (Fig. 4, *i.t.c.*), and are found in lizards and mammals. They form the epicondyles of the humerus, the olecranon of the ulna, the styloid process of the radius, and the tuberosity of the tibia, and it seems very likely that many of the late-appearing centres of mammals, such as those forming the margins of the scapula and ilium and the spines of the vertebrae belong to this class, but this has not yet been determined.

By mistaking the intratendinous centre of the olecranon of *Sphenodon* for an ulnar patella, Parsons (1905) was led to the belief that a sesamoid could join a long bone to form a secondary centre. But it is now becoming more certain that the typical secondary centres, intratendinous centres and sesamoid bones have retained their identity throughout evolution (Haines, 1940).

XVIII. PARALLEL EVOLUTION AND SECONDARY SIMPLIFICATION

Secondary centres have been evolved independently in some fishes, frogs, lizards and mammals, and possibly in birds. Cartilage canals again have been evolved independently in the varanid lizards, birds and mammals and possibly in frogs. Even the details of development may be identical without indicating any near affinity, for instance the formation of centrifugal canals in varanids and mammals, and the perforating canals of mammals and birds. In fact the development of cartilage canals in the various phyla that possess them constitutes one of the most perfect examples of parallel evolution that has ever been described.

Again, epiphysial structure offers excellent illustrations of secondary simplification. There seems little doubt that the urodeles are all descended from ancestors which had

well developed cartilage columns and endochondral bone, that the ancestors of *Phyllodactylus* had secondary centres of ossification as have other lizards, or that the rat and mouse have lost a cartilage canal system. In each case a more complex structure has given rise to a simpler structure, which might mislead, and in some cases has misled, students into the belief that the structure in question was primitive.

XIX. SUMMARY

1. The most primitive type of epiphysial mechanism known is found in bony fishes, and from this type all others are easily derivable. The cartilaginous epiphyses contain a mass of undifferentiated cells, a growth zone of flattened cells, and a zone of hypertrophied cells derived from them. Endochondral bone and marrow are well developed. Temporary cessation of growth may occur with loss of differentiation in the growth zone and the formation of a closing plate of endochondral bone shutting off the marrow from the epiphysial cartilage.

2. In some modern fishes, Chondrostei and Dipnoi, endochondral bone and marrow have been lost, in others a secondary centre of calcification may be found in the epiphysis, but these changes are peculiar specializations.

3. In primitive tetrapods, including all typical early fossil forms and the living Crocodilia and Chelonia, the cells of the growth zone are arranged more or less regularly in columns, and the endochondral bone, guided by these columns, again has a regular arrangement.

4. Of the modern amphibians the Urodela have lost the regular arrangement of the cells of the growth cartilage, and have reduced the amount of their endochondral bone, while the Anura have developed a peculiar match-head type of epiphysis with a calcified secondary centre.

5. In *Sphenodon* a large calcified secondary centre is developed between the articular cartilage and the growth cartilage. By separating these cartilages it allows each to adopt the most advantageous position, the one for the formation of the joint surface, and the other for directing the arrangement of the trabeculae of the endochondral bone.

6. All other groups are specialized in one direction or another. Bony secondary centres which strengthen the epiphyses are found in typical Lacertilia except in some small forms which have lost them, in the tibia of Aves and in Mammalia. Cartilage canals, developed primarily for the nutrition of the cartilaginous epiphyses, are found in Varanidae, Aves and Eutheria, and possibly in Anura, while the Monotremata have a very peculiar cartilage canal system whose development is not yet understood. These features are the result of very detailed parallelisms in evolution.

7. Intratendinous centres of ossification, formed by ossification of tendons where these are inserted into epiphysial cartilage, are distinguished structurally from typical epiphysial centres.

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KEY TO LETTERING

In all diagrams bone is shown black, cartilage white, and calcified cartilage stippled.

<i>a.f.t.</i>	articular fibrous tissue	<i>l.t.</i>	longitudinal trabecula
<i>b.e.</i>	bay of erosion	<i>m.p.</i>	marrow process
<i>b.m.</i>	bone marrow	<i>m.p.zry.c.</i>	marrow process of secondary centre
<i>br.c.</i>	branch of cartilage canal	<i>m.s.i.c.</i>	marrow space of intratendinous centre
<i>br.obl.c.</i>	branch of obliterated canal	<i>nut.can.</i>	nutrient canal
<i>cal.m.zry.c.</i>	calcified matrix of secondary centre	<i>nut.i.t.c.</i>	nutrient canal of intratendinous centre
<i>c.can.</i>	cartilage canal	<i>obl.c.</i>	obliterated canal
<i>c.clm.z.g.</i>	cartilage column of zone of growth	<i>o.cl.s.</i>	osteoclast of shaft
<i>c.clm.z.h.</i>	cartilage column of zone of hypertrophy	<i>o.cl.zry.c.</i>	osteoclast of secondary centre
<i>cen.can.</i>	centrifugal canal	<i>p.b.s.</i>	periosteal bone of the shaft
<i>c.l.p.</i>	cellular layer of periosteum	<i>p.c.t.</i>	perichondral tissues
<i>c.m.z.h.</i>	calcified matrix of zone of hypertrophy	<i>per.c.</i>	perforating canal
<i>co.c.</i>	core of cartilage	<i>per.g.c.</i>	perforation of growth cartilage
<i>con.c.</i>	cone of cartilage	<i>p.z.</i>	peripheral zone
<i>c.p.e.b.</i>	closing plate of endochondral bone	<i>t.e.b.</i>	trabecula at end of bay
<i>c.t.l.s.</i>	connective tissue between lappet and shaft	<i>t.p.b.</i>	trabecula of periosteal bone
<i>e.b.</i>	endochondral bone	<i>t.s.b.</i>	trabecula at side of bay
<i>e.b.can.</i>	endochondral bone lining canal	<i>t.t.</i>	transverse trabecula
<i>e.b.per.c.</i>	endochondral bone in perforating canal	<i>u.c.</i>	uneroded cartilage
<i>e.b.zry.c.</i>	endochondral bone in secondary centre	<i>z.a.c.</i>	zone of articular cartilage
<i>en.zry.c.</i>	entrance of tissues forming secondary centre	<i>z.f.c.</i>	zone of flattened cells
<i>f.l.p.</i>	fibrous layer of periosteum	<i>z.h.c.</i>	zone of hypertrophied cells
<i>g.m.p.b.</i>	growing margin of periosteal bone	<i>z.u.c.</i>	zone of undifferentiated cells
<i>is.m.gr.c.</i>	isolated mass of growth cartilage	<i>1ry.t.</i>	primary trabecula
<i>i.t.c.</i>	intratendinous centre	<i>2ry.t.</i>	secondary trabecula
<i>lap.</i>	lappet	<i>2ry.c.cal.</i>	secondary centre of calcification
<i>lig.cr.</i>	cruciate ligament	<i>2ry.c.os.</i>	secondary centre of ossification

BODY TEMPERATURE IN POIKILOTHERMAL ANIMALS

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I. INTRODUCTION

Heat is a form of energy. It can be *transformed* into other forms of energy—chemical, kinetic, electrical, radiant, etc.—and can be obtained by transformation from them. It can be *transferred* from one place to another directly by conduction and by convection, or indirectly after transformation. Animals have no sense organs capable of registering a *quantity of heat*. Their sensory equipment registers differences of *temperature*, and temperature is the energy level of heat.

The principal factors tending to raise or lower the temperature of an animal's body are conduction and convection of heat, and radiation, while chemical transformations tend to raise it and evaporation of water tends to lower it. In most animals the resulting temperature generally differs little from that of the environment; these are the poikilotherms. The focus of interest is the extent and causes of such divergences from environmental temperatures as do occur. In a relatively small number of animals, the birds and mammals or homoiotherms, the body temperature is high, fairly constant and fairly independent of the environment; the focus of interest in this group is therefore the mechanism by which this high temperature is maintained and the extent and causes of divergences from normal body temperature (DuBois, 1937; Burton, 1939).

II. AQUATIC POIKILOTHERMS

For the poikilotherms we may start by assuming a body temperature identical with a uniform temperature of the effective environment. Under these conditions no direct resultant transfer of heat could take place, but body temperature would tend to rise owing to the evolution of heat in metabolism and it might tend to fall because of evaporation. Aquatic animals cannot lose water by evaporation, and exchanges of water in other ways involve only small quantities of heat, so such animals have no means of acquiring a body temperature below that of the surrounding water. Consequently we should expect the body temperatures of such animals to be above the temperature of the surrounding water. As soon as the body temperature rises, however, heat can be lost by conduction to the water and carried right away by convection. The greater the rise of temperature, the greater the rate of conduction; at a certain point the metabolic

heat will all be carried away as quickly as it appears, so that the body temperature will reach a steady value corresponding to a steady metabolic rate. The question then arises of how much this body temperature differs from the water temperature.

Now water has unusual properties in keeping temperatures constant. Its specific heat is higher than that of any other common substance, so that a large amount of heat is required to warm it; being a fluid it makes perfect contact with the external surface and so easily acquires heat from the animal; and what it lacks in conductivity is made up for by its mobility in convection. These considerations lead us to expect only small differences between body and environmental temperatures in water.

On the animal side of the equation the metabolic rate has to be considered; other things being equal, a high metabolic rate should result in a high body temperature. It would be very far from true to postulate an evolution of heat proportional to the weight (W) of the animal. Within groups of animals the metabolism as measured by oxygen consumption is sometimes more nearly proportional to the surface area ($W^{\frac{2}{3}}$) (Rubner, 1928) or to some power of the weight less than unity ($W^{\frac{1}{3}}$, Kleiber, 1932; $W^{0.78}$, Benedict, 1938). At any rate small animals generally have a higher rate of heat production per gram of body weight than large animals. There is, however, no simple rule connecting size and intensity of metabolism for all phyla of animals; sluggish animals, like lamellibranch molluscs, have a much lower respiratory rate than active animals like insects.

Apart altogether from metabolic rate small size is in favour of rapid equalization of internal and external temperatures, while large size is in favour of a temperature above that of the environment. That is to say, the heating effect of high metabolic rate in small animals is opposed by the more effective cooling due to the large surface area per gram. A bulky animal may be hot inside and cold near the surface, the cold environment cooling the superficial tissues while leaving the interior quite warm. Thus Bazett (1927) recorded that a thermocouple thrust into the muscles of the human thigh shows that the temperature at a depth of 2-3 cm. can be lowered by 5.7°C . by cold applications to the skin, the skin surface being then considerably cooler than the muscles, of course, while the 'body temperature' measured in the mouth or rectum remained at the usual high level. Thus considerable gradients of temperature can occur if the animal is large. In small animals, on the other hand, the distance from the centre to the surface is small and an equal steepness of gradient implies a correspondingly smaller excess of temperature at the centre; conversely, a difference of temperature as large as that found in the human case would require a very high production of heat indeed.

An efficient circulatory system tends to reduce temperature gradients in the body, though as we have just seen does not eliminate them. For example, Pearse & Hall (1928) recorded the changes of temperature occurring in the coelom of a turtle weighing 278 g. on transfer from water at about 10°C . to water at freezing-point. The temperature fell more rapidly in a living turtle than in a dead one, simply owing to the heat carried in the blood circulating in the former, and in spite of the fact that the living turtle was also producing metabolic heat.

Although radiation from the sun is known to be important to land animals, it has not been shown by direct evidence to affect the body temperatures of aquatic animals. Gelei (1928) has described changes in the dark pigment lying on the air sacs of the phantom larva of *Corethra*. This pigment appears to be more extensive at 2° than at 17°C ., and Gelei postulated a greater absorption of heating radiation at the lower

temperature. Quite apart from the fact that water stops and absorbs a good deal of the radiant energy of sunlight it seems very unlikely that an animal as small as *Corethra* could have a body temperature measurably above that of the surrounding water.

Measurements of body temperatures of aquatic poikilotherms seem to be rare. Simpson (1908) found no differences between the water and the body greater than 0.12°C . for echinoderms and crustaceans (*Cancer*) and 0.7°C . for a large fish like the cod (*Gadus morrhua*) weighing up to about 10 kg. These fish had been struggling violently for about 2 min. before their temperatures were taken, while being hauled up from a depth of 90 m., so the heat produced in the muscles had probably raised the temperature above its normal value. Normally, the difference between water and body temperatures is probably even less than 0.7°C . As long as the water temperature was not rising Simpson found no single animal cooler than the water around it.

Nielsen (1938), using a thermocouple stuck into the dorsal muscles of the fish *Lebistes*, could detect no difference greater than the variation in the temperature of the water (0.1°C .). When the fish was transferred from 17.7 to 33.1°C ., the temperature in the muscles hardly differed from that of the water after 1 min. Rogers & Lewis (1916) found no difference greater than 0.07°C . in nine measurements of the stomach temperature of goldfishes weighing 11–26 g., and even this difference, since it represented a body temperature lower than water temperature, must have been due to lag in adjustment. In fishes the respiratory current of water which is being continually passed over the gills, very close to large amounts of circulating blood, is probably important in ensuring rapid equalization of temperatures.

Measurements made by Rogers & Lewis (1914, 1916) on the earthworm immersed in water gave similar results. At the steady state the difference was less than 0.05°C .; 2 min. after the water temperature had been changed by 10°C ., the temperatures of worm and water differed by less than 0.05°C . Rogers & Lewis (1916) also worked on *Anodonta*, the fresh-water mussel, weighing 200–250 g., the axolotl, *Amblystoma punctatum*, and the salamander, *Diemyctylus viridescens*, with similar results. For the last species, out of seventy-three measurements at water temperatures between 16 and 35°C . twenty-seven gave no difference, fourteen gave a body temperature higher by a maximum of 0.02° (average 0.01°), and in the remaining thirty-two the body temperature was lower by an average of 0.02° (maximum 0.26°), the average difference for the whole set of experiments being of the same order of magnitude as the limit of recording (0.002°C .).

Kestner & Plaut (1924) quote older data tending to show large differences between water temperature and fish body temperature up to 8°C . In the older papers, however, lack of experimental details prevents us from being satisfied that proper precautions were taken (Tigerstedt, 1910). With ailing fishes, in which the respiratory current has ceased, higher body temperatures may occur for short periods.

There are, therefore, only slight errors involved in regarding small aquatic poikilotherms as having substantially the same temperature as the surrounding water. The errors might be greater if the same approximation were used for larger animals like sharks or for animals which lie so near the surface of the sea as to be affected by radiation from the sun. Simpson (1908) quotes work done by John Davy in 1816 on the bonito (*Thynnus pelamis*) and the tunny (*Thynnus vulgaris*) which suggests that these large fishes may have parts of the body as much as 10°C . warmer than the surrounding water (see below, pp. 309–10).

The work of Baldwin (1925) appears to provide an exception to the general conclusion reached above. Using two species of turtles, *Chrysemys marginata* and *Chelydra serpentina*, weighing 265–712 and 152–1725 g. respectively, this author recorded rectal temperatures while the animals were immersed in water. When the water temperature was kept steady at 32° F. (0° C.) for 2½ hr., the rectal temperature became steady at 41° F. (5° C.). The excess of body temperature was attributed to the evolution of additional metabolic heat. This is sufficiently exceptional amongst modern results and sufficiently interesting to be worth while repeating.

III. TERRESTRIAL POIKILOTHERMS

With land poikilotherms the situation is much more complicated. In the first place it is only in laboratory experiments that there is a reasonably constant and uniform environmental temperature. Although gradients of temperature do of course occur in water, they are relatively stable and are not steep compared with gradients which can occur in air. On the other hand, on land radiation from the sun or to the night sky sets up a patchwork of differences of temperature. This leads in turn to air convection currents which distribute the heat to some extent, but the specific heat of air is too low to allow the air to level out temperature gradients at all quickly. Incidentally a thermometer itself forms part of the patchwork of temperatures, and consequently air temperature is more difficult to measure than is generally realized. The temperature map of the land is further complicated by evaporation and deposition of water. Whether one is dealing with a whole continent or a piece of ground small enough to be covered by a penny there is frequently no single temperature which can be taken as accurately representative of the environment except under carefully controlled experimental conditions.

In the second place the animal body itself is an object which may have a temperature different from that of the nearest other object and from the air around it. The air is a good insulator when stationary and does not carry heat very effectively. It does transfer some heat, however, and heat exchange occurs at the animal's surface in other ways, so that the surface of the animal may be measurably cooler than the deeper parts. Consequently in the animal itself there is no longer one temperature which is truly representative of the whole body. The situation is further complicated by the cooling effect of evaporation from the animal itself and by radiation.

Land poikilotherms belong mainly to three phyla—molluscs (gastropods), arthropods (excluding most Crustacea and the aquatic insects), and the Amphibia and reptiles among the vertebrates. No modern information appears to be available about body temperatures of land gastropods; since these animals metabolize relatively slowly and keep to moist and shady places, where evaporation and radiation affect them little, it seems unlikely that they will get much warmer or cooler than their surroundings.

IV. INSECTS

1. *Evaporation*

The earlier work on insects was reviewed by Bachmetjew in 1899 when most of the complicating factors other than radiation were already well known. He himself showed that in an air temperature of 17.5° C. the moth *Saturnia pyri* could raise the temperature

of its thorax by muscular activity from 19.8 to 24°C . in $2\frac{1}{2}$ min. It then took $8\frac{1}{2}$ min. for the temperature to fall to 18.7°C ., the moth being at rest. This contrasts sharply with the rapidity of adjustment of body temperature of even much larger animals in water. Bachmetjew used thermocouples, and it is clear that the lag was in the animal and not merely in the thermometer (see also Isserlin, 1902; Uvarov, 1931).

Necheles (1924) used a mercury-in-glass thermometer thrust through the anus up into the thorax of the cockroach, *Blatta orientalis*. At temperatures below 10°C . the body temperature was a little above the surrounding air temperature, perhaps a degree higher at 5°C . The drying power of still air at low temperatures is never very great, so evaporation could not affect the body temperature much, radiation was not in question as an important disturbing factor, and the slight excess of body temperature must have been maintained by the heat of metabolism. Up to about 23°C . body temperature was

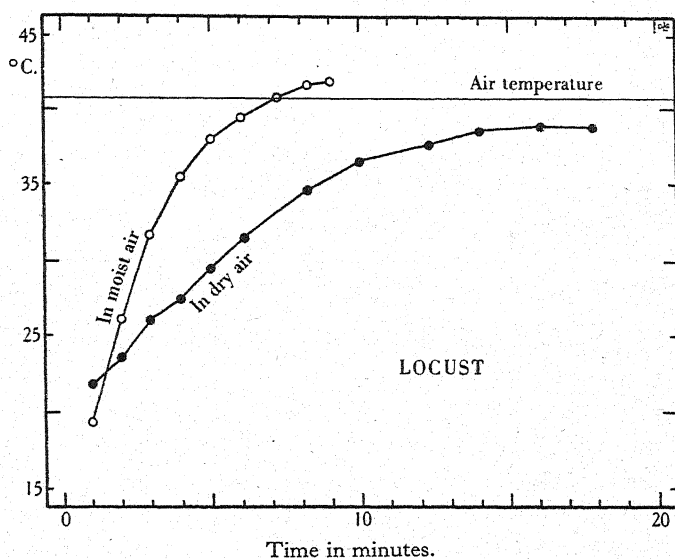


Fig. 1. Body temperature of a desert locust, *Schistocerca gregaria*, in moist air and in dry air. The air temperature is indicated by the upper line (41.5°C .). (After Bodenheimer, 1930.)

effectively identical with air temperature. Above about 25°C . the body temperature depended on the humidity of the air, so that in moist air it was very slightly above and in dry air below the temperature of the air. At 30°C . the difference in dry air was about 3° and at 40°C . it was about 5° . Since the extremes of the environmental temperature were thus somewhat moderated within the body—at any rate in dry air—Necheles concluded that the cockroach shows a rudimentary regulation of its body temperature. Using similar methods on the larger cockroach, *Periplaneta americana*, Mellanby (1932) also found the lowering of body temperature above 40°C . in dry air and not in moist air. Bodenheimer (1930) found that the body temperature of the desert locust, *Schistocerca gregaria*, at about 40°C . was 3 or 4°C . lower in dry air than in moist (Fig. 1). Koidsumi (1935) obtained a similar result for the grasshopper, *Gastri-margus transversus*, at 30°C . (Table 2, p. 305). These three insects are comparable in size, and both Bodenheimer and Koidsumi used thermocouples for measuring tem-

perature, the latter author giving an extensive series of data. A steady body temperature below that of the air could only be maintained in dry air.

It is worth noticing that in Koidsumi's data in Table 2 (p. 305) the cuticle temperature is always lower than the internal body temperature. If the heat exchange takes place mainly at the surface and not in the tracheae (evaporation), it must always be so at the steady state. If the cuticle were the warmer, heat would flow in, the metabolic heat could not flow out against the temperature gradient, so the internal temperature would rise and the steady state would not therefore have been attained.

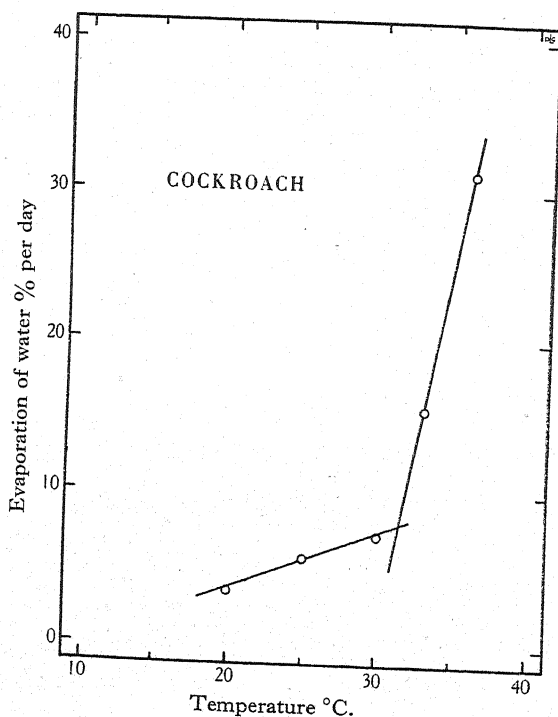


Fig. 2. Rate of evaporation of water from the cockroach *Blatta orientalis* expressed as a percentage of the original weight, at a saturation deficiency of 20 mm. Hg and various temperatures. Note the break in the curve just above 30° C., corresponding to the melting-point of the fatty layer on the cuticle. (After Ramsay, 1935, from Gunn, 1933.)

There is no doubt then that in dry air at higher temperatures the body temperatures of these large insects can be kept below air temperature for a certain time by evaporation of water. This does not in itself constitute regulation in any biological sense, for the same thing happens to a piece of wet rag. Indeed, Koidsumi's temperature measurements of the same insects immediately after they had been killed with cyanide showed that the lowering of temperature occurred in much the same way as it had when the insects were alive. *Gastrimargus* actually cooled slightly more when dead than when alive, presumably owing to the reduction of metabolic heat production in the dead grasshopper.

The position is somewhat more complicated with the cockroach, for the evaporation of water in dry air does not simply rise steadily with rising temperature as it does from a wet rag; round about 30° C. (Fig. 2) it accelerates out of all proportion to the drying power of the air (Gunn, 1933, 1935; Ramsay, 1935). This is the highest temperature at

which a cockroach will become quiescent if lower temperatures are available to it, the upper limit of its preferred temperature (Gunn, 1934, 1935). According to Ramsay (1935) the acceleration in evaporation is due to the melting (or other change of state) of a thin fatty layer on the outside of the cuticle and the consequent increase in permeability of the body wall to water vapour. Has this fatty layer the function of permitting water to evaporate fast enough at higher temperatures to lower the body temperature? Has a fatty layer of these properties survival value for the cockroach? Unfortunately, nothing is known about it except for the cockroach. It is probably true to say that in most insects the evaporation of water from the tracheae increases rather rapidly with rising temperature, for increasing carbon dioxide production in metabolism causes more frequent spiracular opening, and this allows water vapour to escape to an increasing extent (Buxton,* 1932; Mellanby,* 1935). In this case, however, the water lost is lost unavoidably and may be regarded as the price paid by the insect for continuing to get rid of its own carbon dioxide and to receive an adequate supply of oxygen. The drying power of a space with a given water content rises with rising temperature, but this is a purely physical factor affecting all water. On the other hand, no other function for a fatty layer melting at just this temperature has been suggested. It may be purely accidental that it has this melting point at the upper limit of preferred temperature, and this question cannot be decided without comparative work on other insects. Further speculation is not helpful.

In any case the acceleration of desiccation appears to occur in the same way in dead animals (Ramsay, 1935), so it hardly seems justifiable to place this kind of 'regulation' side by side with the active secretion of sweat, dilatation of peripheral blood vessels and so on which occur in mammals. The lowering of body temperature is of only limited value to the insects. For example, a 24 hr. exposure to an air temperature of 37° C. is more often fatal to both *Periplaneta americana* and *Blatta orientalis* in dry air than it is in moist. The loss of water kills when the temperature does not. On the other hand, in a 1 hr. exposure moist air is fatal to both species at 43° C. and dry air is not (Gunn & Notley, 1936). The evaporation and the consequent cooling of the body below air temperature is useful during a short exposure but harmful during a longer one, if water is not available for drinking.

In this matter the size of the animal is important. The available water is a function of the weight, but the heat taken in when the body is cooler than the air is a function of the surface area. This heat and the heat of metabolism must be dissipated by evaporation if the body temperature is to be kept below the air temperature. Consequently a small animal is under a grave disadvantage in keeping its body temperature down, for the water available soon becomes exhausted (Mellanby, 1932). The evidence available suggests that in nature the locomotory behaviour of insects is a far more efficient protection against high temperature than any lowering of body temperature by evaporation (Fraenkel & Gunn, 1940; see also below, pp. 306-8).

There is no doubt that body temperatures in insects can be lowered by evaporation of water, but this is not in itself evidence of biological regulation of body temperature. It would be evidence against biological regulation if it could be shown that as temperature rises water loss rises simply according to physical rules, without the intervention of new factors or the unexpected accentuation of factors already acting at lower temperatures.

* *Biological Reviews.*

Certain authors have recorded the progress of change in body temperature when the surrounding air temperature is suddenly altered; the curve obtained on plotting temperature against time appears to conform reasonably well with what is expected from Newton's law, the rate of transfer of heat being approximately proportional to the difference of temperature (Bodenheimer & Samburski, 1930; Koidsumi, 1935). It is therefore argued that there is no regulation, although the final body temperature is lower in dry air than in moist. The conclusion is justified only if regulation is conceived of as necessarily intruding suddenly, like the sudden production of beads of sweat in man. If some kind of temperature regulation were to intrude suddenly during rising temperature, it might produce a discontinuity in the curve of body temperature plotted against time. But if rise of temperature were to lead to a progressive—not sudden—rise in some kind of active sweating in insects, it is quite possible that the temperature curves (Fig. 1, p. 297) would still be not only smooth but also tolerably like the Newtonian curve. These curves are not therefore evidence against biological regulation. Apart, however, from the case of the cockroach dealt with above, there appears to be no evidence in favour of biological regulation of body temperature in insects by evaporation of water.

There is a certain amount of information tending to show that insects can gain water from saturated and nearly saturated air (Wigglesworth, 1939), but this process is always too slow to produce an appreciable heating effect.

2. Radiation

The importance of direct radiation from the sun in warming insects appears to have remained almost unnoticed until recently (Buxton, 1923), and even now no very extensive or systematic work has been done. The sun's rays pass through the air without warming it very much, and they warm the ground and other solid objects much more. While in full sunshine an insect may thus be measurably warmer than the air around it. Now different kinds of surface absorb radiation to different extents, and other things being equal, a black animal should therefore warm up more quickly in sunshine than a white one. Rücker (1933*a*) measured the proportion of the energy in the visible part of the spectrum of standard sunshine which was absorbed and converted into heat by the elytra of various beetles. For *Compsus niveus*, a chalky white scaly form, it was 26 %, while for the black *Silpha obscura* it was 95 %.

Unfortunately the matter is more complicated than this; a considerable proportion of the energy in the radiation from the sun is in the infra-red part of the spectrum. With the sun at middling height, for example, Krüger (1929) quotes the following proportions: about 60 % of the total radiant energy in the infra-red, about 40 % in the visible and about 1 % in the ultra-violet. Consequently the colour of a surface gives only a very incomplete indication of the extent to which the surface reflects or absorbs the radiation, particularly in that it gives no indication at all of what happens to the infra-red. In any case, a colour merely indicates that *some part* of the radiation of that part of the visible spectrum is reflected instead of being transformed into heat. For example, the blue-green beetle, *Hoplia farinosa*, reflects only 40 % of the blue-green light falling on it, while the red beetle, *Melasoma saliceti*, reflects less than 20 % of the red light (Rücker, 1933*a*).

Moreover, quite apart from reflexion, the radiation emitted by an animal's body in

virtue of its own temperature and the nature of its surface is entirely in the infra-red and is therefore invisible. Deighton (1933) has discussed this question in relation to comparisons between negroes and caucasians in the tropical sun and has pointed out that what the melanin in the skin does is to prevent the penetration of the radiation into the deeper layers. Apart from this no reliable difference in the heat economy of negroes and 'whites' has been demonstrated, in spite of a number of efforts, and the unselective reflexion of a certain small proportion of the visible rays by the lighter skin seems to be of little importance. The radiation from the human body itself has its maximum in the very long infra-red at a wave-length of 9.44μ and half or three-quarters of the heat dissipated may leave the body as infra-red radiation. The radiating properties of the surface of the body, in virtue of its own temperature, may thus be of considerable importance in determining the resultant effect of radiation on body temperature, as long as there are surroundings at lower temperatures.

Some measurements indicating the relative importance of the various wave-lengths have been carried out by various members of the Vienna school. The colour of a beetle gives little indication of the ability of its surface to absorb radiation. Thus, for the artificial radiation used by Duspiva & Cerny (1934), the dark brown elytron of *Rhynchophorus palmarum* absorbed the visible radiation ($470-670\mu\mu$) almost as well as the standard black surface (94%), but it absorbed only 64% of the red to medium infra-red ($640-3000\mu\mu$). The black *Steraspis squamosa* and the brown *Rhizotrogus aequinoctialis* behaved almost identically towards the visible, absorbing 75 and 77% respectively, but the former absorbed 59% in the infra-red and the latter only 35%. Still, pigmentary colour has some importance in determining the amount of radiation absorbed, but shiny surfaces and interference colours have at most subordinate effects (Duspiva & Cerny, 1934; Rücker, 1933a, 1934).

In the infra-red itself reflexion of the various wave-lengths varies, though not quite as much as it does in the visible. If we could see colours in the infra-red as we can in the visible spectrum, then insects would show considerable variation in infra-red colours. Rücker (1934) has shown that the elytron of *Carabus hispanus* reflects 35% of the incident radiation at the wave-length 1.1μ , 17% at 2.15μ , and 46% at 3μ , while at these wave-lengths the wing of *Pieris brassicae*, the cabbage-white butterfly, reflects 69, 55 and 35% respectively.

The radiation from the sun ends at about 3μ . Still longer infra-red rays up to 10μ are emitted by objects on the earth's surface and these can be transformed into heat. Hot stones and even the bodies of animals themselves emit these rays, but practically nothing is known about them in relation to the body temperatures of poikilotherms.

In describing the effects of evaporation on body temperature it was possible to quote experiments in which radiation was probably negligible. No experiments appear to have been done on the effects of radiation when evaporation is not an effective factor, and in general the existing data show only in broad outline what sunshine can do.

The importance of colour in determining the final body temperature has been investigated directly by several authors. The grasshopper, *Calliptamus (Kripa) coelesyriensis*, occurs in Palestine in two forms, a buff or light sandy-coloured one and a dark chocolate or nearly black form (Buxton, 1924). When exposed to the sun tied to a wire frame above the sand surface the dark one became and remained $4-5^{\circ}\text{C}$. warmer than the lighter

one (Fig. 3). Both were warmer than the air and cooler than the underlying sand. The external humidity conditions were the same for the two individuals, but of course the difference of body temperature might have been affected by different rates of evaporation from the two varieties or different rates of metabolism. Hill & Taylor (1933) found a similar difference of body temperature of gregarious (black) and solitary (pale green or buff) locusts in sunshine, but did not control this result by recording the body temperatures in the absence of intense radiation.

The speed at which radiation can raise body temperatures is very striking. Krüger (1931) fastened a grasshopper, *Gomphocerus sibiricus*, on to a white stone in full sunshine in the mountains (2050 m.); the air temperature was 22.2° C. In 45 sec. the body temperature rose from 26.6 to 40.3° C. Part of the heating effect would presumably come from the stone. Again, Bodenheimer (1930), working with the desert locust, *Schistocerca*

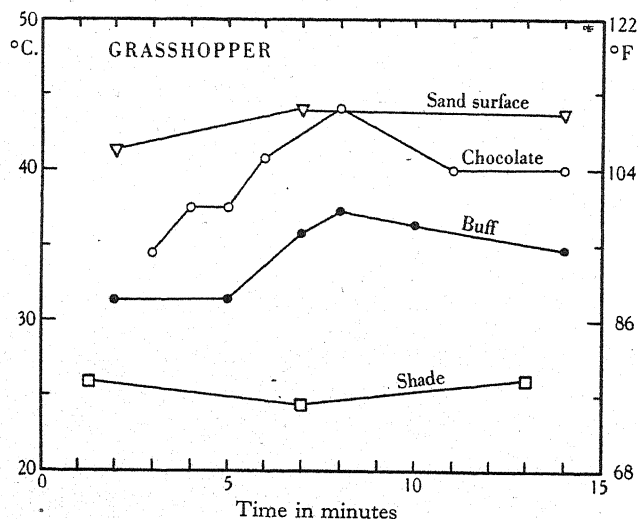


Fig. 3. Body temperatures, taken with thermocouples, of two colour varieties, chocolate and buff, of a grasshopper, *Calliptamus colesyiensis*, exposed to sunshine. The sand surface and shade temperatures are also shown. The temperature of the black bulb in vacuo was 64° C. (115° F.). (After Buxton, 1924.)

gregaria, recorded at 8.00 a.m. a body temperature of 36.2° C. in the sunshine, the air temperature being 23.9° C. At 8.10 a.m. the animal was in the shade, the air temperature having fallen by 0.9° C., but the body had cooled by 10° to 26.2° C. Later, at 9.50 a.m., after strong sunshine, the body temperature was 42.0° C. and the air only 26.2° C. The soil surface was warming up more rapidly than the air but not so quickly as the locust. Kennedy (1939), working in the Sudan, used a thermocouple to measure the body temperature of a locust in shade and in sunshine. With an air temperature of about 30° C., when the shade was removed body temperature rose by 4° C. in the first minute and by 7° C. in 5 min. Such changes of temperature had most interesting and unexpected effects on the locomotory activity of locusts. A rise of 7° C. in body temperature produced a considerable increase of activity lasting only a few minutes, while a fall of the same magnitude produced a greater increase of activity lasting for over 20 min.

These changes of temperature are not uncomplicated by evaporation and by heat of metabolism, but they are changes which would not have occurred except for the

sunshine. A very great error would thus have been made if it had been assumed that the temperature of the body was the same as that of the surrounding air. There is a very striking contrast between such a case and that of a similar animal in water.

3. Metabolic heat

Reference has already been made to the fact that metabolic activity always results in the appearance of heat and so tends to raise body temperature. Other things being equal, the extent to which the body temperature can rise should depend on the relation between the weight of heat-producing tissue and the surface area through which heat can be lost. It happens, however, that in comparable animals of different sizes the rate of oxygen consumption per unit weight is not the same but is higher in the smaller animal; for example, in three species of cockroaches the oxygen consumption per animal is more nearly proportional to the surface area than to the weight (Gunn, 1935). In this case, then, the proportionately smaller surface area per unit weight of the largest species (912 mg.) should not lead to a body temperature any higher than that of the smallest species (47 mg.). The similarity of temperature relations in these three species

Table 1. *Thermocouple measurements of body temperature of a sphingid moth, the air temperature being the same as the initial body temperature (Dotterweich, 1928)*

Temp. at start of fluttering °C.	Temp. at taking off in flight °C.	Temp. during flight °C.
9	34.0	37.8
12	35.5	38.6
15	33.8	—
22	35.2	41.5

of cockroaches is actually still greater than this, for the rate of evaporation of water in dry air at 30° C. is closely proportional to the rate of oxygen consumption, so that any cooling due to evaporation must run parallel to the metabolic heating and tend to neutralize it.

It is not possible to make a precise general rule about the relation between size and rate of heat production in insects generally. Factors other than size are probably of much greater importance in determining the rate of heat production. It has already been pointed out, for example, that the increase in muscular activity resulting from taking to flight causes a considerable rise in body temperature. Under appropriate conditions the body temperature must always be above that of a uniform environment, even in the absence of any special activity. These conditions are that the environmental temperature should be really uniform so that there can be no resultant transfer of heat through radiation or conduction, and that the air be saturated with water vapour so that no cooling by evaporation can occur. Metabolic heat cannot then leave the body until the body temperature has risen, however slightly. But under real conditions of low activity and not quite saturated air the excess of body temperature may be quite slight (Table 2, p. 305).

When there is considerable activity, on the other hand, the situation is quite different. When an insect takes to flight its oxygen consumption, and therefore its heat production, may be multiplied 37 times (*Deilephila*, Kalmus, 1929) or hundreds of times (honey-bee,

Kosmin, Alpatov & Resnitschenko, 1932), and this causes a considerable rise in temperature. The rise in body temperature in turn increases the basal metabolism and, more important, allows the wing muscles to work still faster and so further increase the production of heat. Some insects cannot fly at low temperatures, but by fluttering the wings they can raise the body temperature progressively in this way until flight becomes possible. Dotterweich (1928) has shown that a sphingid moth (species not mentioned) does not fly until its body temperature is above 30°C ., and if the environmental temperature is low the temperature necessary for flight is reached by means of wing fluttering (Table 1). The moth was suspended in an air thermostat by a thermocouple thrust into the middle of the thorax. The duration of fluttering before flight begins is long or short according as the initial temperature is low or high respectively. Bodenheimer (1934) has reviewed the subject; he himself found that for the moth, *Macroglossa stellatarum*, the body temperature at taking off in flight was 21.5°C . ($19.7\text{--}23.7^{\circ}\text{C}$.), and that flight ceased when the temperature rose to 26.6°C . ($24.0\text{--}32.6^{\circ}\text{C}$.). In the social Hymenoptera muscular activity is of great importance in the regulation of the temperature of the nest (Uvarov, 1931; Himmer, *1932).

4. Other factors

There remain two factors, conduction and convection, which always tend to equalize the body temperature and the temperature of the immediate surroundings. Radiation may do this too, of course, but primarily radiation is the means by which temperatures are influenced at a distance. So far as we are here concerned, conduction and convection are complementary processes. Heat is passed by conduction between the insect's surface and the thin layer of air in contact with it, while convection assists in the prior or later movements of heat towards or away from this thin layer. There must also be some conduction of heat between an insect and any solid surface with which it may be in contact, but nothing is known about the magnitude of any such exchange. In air, if convection can be largely prevented, conduction is responsible for little heat transfer; stationary air is a good heat insulator. The function of insulation has been attributed to the subelytral air space of certain beetles (Franz, 1930).

It is well known that hair in mammals and feathers in birds act as heat insulators largely because they trap a stationary layer of air. It does not follow that the thick coat of scales found in certain moths acts in the same way; doubt arises because of the difference in size relations. A layer of material added by an animal to its true body surface increases the contact area with the surroundings; the percentage increase due to a given thickness of coat is far greater for a small animal than a large one. Indeed, it is easy to show that for a cylindrical body the *absolute* increase is the same whatever the radius of the body. It is well known to engineers that the advantage of having a poorly conducting layer added to the surface of a thin pipe may be quite outweighed by the disadvantage of having a larger contact surface with the surrounding freely moving air; in fact, the addition of 'insulation' may actually speed up the heat exchange. It should not be difficult to find out whether the scales of moths help or hinder heat exchange, but it has not yet been attempted.

Some rough experiments carried out by Weiss (1914) on empty cocoons of the silkworm, *Bombyx mori*, and other moths are suggestive and should be elaborated with

* *Biological Reviews*.

suitable modifications. He showed that a thermometer bulb inside the cocoon changed in temperature more slowly than a fully exposed one. This case is not fully comparable with that of the heavily clothed moths, for in the latter the covering makes heat-conducting contact with the body at the base of each scale, while in the cocoon there may be very few points of contact.

No information is available about the specific thermal conductivities of insect tissues. Owing to the sluggish blood circulation, considerable differences of temperature may occur between abdomen and thorax, and it is therefore customary to take the temperature in the thorax, where the greatest muscular heat is developed, and to regard that as the typical value.

5. Energy balance-sheet

If an animal is kept in constant environmental conditions, its body temperature should soon become fairly steady. Variations always occur in reality, mainly because the animal's activity varies and so causes variation in heat production and evaporation. Ideally there

Table 2. *Heat balance-sheet of the grasshopper, Gastrimargus transversus (av. weight about 2 g.). Quantities of heat are in g.cal. per kg. per hr. (Koidsumi, 1935)*

External temp. ° C.	Rel. hum. %	A Metabolic heat produced	B Heat lost by evaporation	C Heat lost by radiation and conduction	D Unexplained balance A - (B + C)	Av. divergence from temp. of air ° C.	
						Body cavity	Cuticle
10	30	3,100	3,090	0	+ 10	+0.47	+0.32
	60	3,240	2,410	170	+ 650	+0.63	+0.49
	90	3,840	2,000	400	+1440	+1.28	+1.17
20	30	7,380	8,500	0	-1120	-0.38	-0.63
	60	7,490	7,500	0	- 10	+0.44	+0.36
	90	6,940	4,290	180	+2470	+0.47	+0.44
30	30	14,080	17,220	0	-3140	-1.52	-1.80
	60	14,720	14,520	0	+ (200)*	-0.19	-0.35
	90	14,370	9,460	250	+4660	+0.87	+0.64

* There seems to be an error in the author's table in this line. He puts -367.42 here. His figures are given to 0.01 cal. and they are given here to the nearest 10 cal.

should be a steady body temperature corresponding to steady internal and external conditions; in these circumstances the animal must neither be losing nor gaining heat. Gains from metabolism, gains or losses from radiation, conduction and convection, and losses due to evaporation, must then cancel out. Any resultant gain or loss must change the body temperature until a new steady state is reached. Koidsumi (1935) has attempted the construction of a balance-sheet showing these exchanges (Table 2). The heat produced in metabolism was estimated by calculation from the oxygen uptake and the respiratory quotient, which were found gravimetrically. The rate of water loss was also estimated gravimetrically. Loss of heat by radiation and conduction (including convection) was found by means of a compensation calorimeter. The apparatus was not adapted to measure radiation absorbed by the animal nor heat conducted to it from the surroundings, and that explains two of the large negative values in column D. The balances in this column at 90% relative humidity at each temperature are mysterious; taking the extreme

view that they should be included under radiation and conduction, it is still apparent that even in nearly saturated air most of the metabolic heat is taken off by evaporation of water. In marked contrast with the state of affairs in mammals, at middling humidities very little of the heat goes away by conduction or as radiation and the reason for this is clear enough: generally the body temperature of a homoiotherm is considerably above air temperature—if it is not, then radiation and conduction become reduced in importance. On the other hand, in insects body temperature is markedly above air temperature only during high activity or in strong sunlight. When the difference between body and air temperatures is small, then conduction ($=k(T_1 - T_2)$) and radiation ($=K(T_1^4 - T_2^4)$) must also be small; if the difference is large, they too can be large. In Koidsumi's experiments body and air temperatures were close together.

To sum up, we may say that in insects metabolic heat is always tending to warm the body, while the evaporation of water is the main method of losing this heat. Whenever these two factors do not balance, dull radiation and conduction (with convection) tend to equalize body and air temperatures. Exceptionally, very high activity or bright radiation, i.e. sunshine, may raise the body temperature rapidly and considerably, while at high temperatures in dry air evaporation may lower it several degrees.

V. AMPHIBIA AND REPTILES

Amphibia when on land are like terrestrial gastropods and unlike insects and reptiles in having a moist surface. A frog with its skin removed loses water no more rapidly than an intact frog, so that the skin affords no protection to the tissues against evaporation (Adolph, 1932). By contrast a normal lizard loses water much more slowly (1/24th) than a newt, but a skinned lizard dries up almost as rapidly (22/24ths) as a newt (Gray, 1928). The extreme rapidity of evaporation from Amphibia at ordinary temperatures is sufficient to cause a degree of lowering of body temperature which is unknown amongst the insects. Indeed, so rapid is evaporation in dry air that in a current flowing at 1.9 m./sec. the body temperature falls to the wet-bulb temperature (Mellanby, 1941). Table 3 shows the effect clearly and contrasts results from Amphibia and reptiles.

It is well known that Amphibia on land frequent only moist and shady places, and this *behaviour* protects them against desiccation, just as the *structure* of the lizard does; but even in 75% relative humidity there is a significant lowering of body temperature (2.5–3.0° C., Table 3). In fully saturated air at rigidly uniform temperature Adolph (1932) has pointed out that the body temperature of the frog must rise above that of the surroundings, for evaporation is the sole method of losing metabolic heat when body temperature is equal to or below that of the environment. For this reason, and in spite of the fact that the vapour pressure of the tissues must be slightly below that of pure water, frogs cannot gain water (nor therefore latent heat) from the atmosphere. A similar argument would seem to apply to insects and the gain of water reported in certain cases remains mysterious (Wigglesworth, 1939).

Even in conditions of saturation the frog loses one-fifth of its metabolic heat by evaporation, for the air is saturated at its own temperature but not at the slightly higher temperature of the frog's body, while in dry air so much heat is lost in this way that large amounts of heat are taken in by conduction, etc., from the surroundings (Adolph, 1932). No precise information is available about humidities in the natural haunts of

Amphibia, so it is not possible to say more about the body temperatures of these animals in nature.

Krüger & Kern (1924) and Rücker (1933*b*) have measured the permeability of the skins and certain other tissues of some lizards, frogs and insects to various wave-lengths of radiation. The skin of the lizard, an animal which basks in the sun, lets through considerably less radiation than that of the frog, an animal which keeps to shade. Because of this habit the frog does not require to protect its deeper tissues against the intense rapid heating which might occur in sunlight. It is possible that the primary function of superficial pigments in land animals is the protection of deeper sensitive tissues against heating radiation; the transparency of many marine invertebrates would then depend on the fact that the surrounding water provides them with similar protection. No direct measurements of body temperatures of frogs in sunlight appear to have been made, and the effects of colour change have not been investigated from this point of view.

Table 3. *Divergences between body and air temperatures (°C.) for various Amphibia and reptiles at various relative humidities. A plus sign indicates a body temperature above air temperature (after Hall & Root, 1930)*

Species	Relative humidity %				
	7	25	50	75	95-100
Amphibia:					
Salamander, <i>Plethodon glutinosus</i>	-9.2	-6.3	-4.6	-2.5	-0.3
Frog, <i>Rana pipiens</i>	-8.6	-6.7	-4.7	-3.0	-0.1
Toad, <i>Bufo fowleri</i>	-7.3	-5.3	-4.0	-2.5	-0.7
Reptilia:					
Lizard, <i>Sceloporus undulatus</i>	-0.7	-0.7	-0.1	+0.3	+0.6
Horned toad, <i>Phrynosoma cornutum</i>	-0.4	0.0	+0.1	+0.2	+0.4
Turtle, <i>Chrysemys marginata</i>	-0.7	-0.6	-0.5	-0.4	-0.1
Tortoise, <i>Cistudo major</i>	-0.3	-0.2	-0.1	0.0	+0.1
Alligator, <i>Alligator mississippiensis</i>	-0.4	-0.3	-0.1	-0.1	+0.2

The basking habits of some reptiles, especially lizards, are well known. Franz (1930) points out that lizards remain in the shade during the heat of the day and bask at cooler times. He found that at Davos at 14.00 hr. in September, when the air temperature was 9.6° C., after 20 min. in the sunshine two lizards had body temperatures of 29.6 and 33.5° C. The warmer one was darker in colour. Again, on removal to the shade body temperature fell in 25 min. from 40 to 30° C. in the one case and to 25° C. in the other.

Just as the behaviour of terrestrial Amphibia protects them from fatal desiccation, so the behaviour of some reptiles is responsible for the maintenance of a rather high and fairly constant body temperature. The horned lizard, *Phrynosoma modestum*, adjusts the angle between its body and the sun's rays in such a way as to increase the amount of radiation striking the surface when the body is cold, and to decrease it when the body is hot (Weese, 1917). Similar behaviour by locusts has been described by Fraenkel (1930) and further investigated by Volkonsky (1939). Sergeyev (1939) gives a number of microclimatic temperatures from sandy country near Repetek in Turkmenia, U.S.S.R., and shows how a lizard, *Agama sanguinolenta*, and a tortoise, *Testudo horsfieldi*, contrive by sitting in a burrow or moving into the sunshine or under or into a bush to keep the body at a fairly even temperature. Thus the soil surface heats up from 16° C. at 06.00 hr.

to 60° C. at 14.00 hr. The tortoise emerges from its burrow at 24° C. soon after sunrise, and thereafter its body temperature remains above 31° C. and below 37° C. until after sunset.

According to Krehl & Soetbeer (1899) the colour change of the lizard, *Uromastix*, is correlated with body temperature. When put into the sun the previously grey-white animal becomes almost black, which increases the absorption of radiation. When the body temperature reaches 41° C. the skin becomes light again and so presumably retards further temperature rise. When returned to the shade the lizard goes dark again; the authors inferred that this darkening helped to conserve the heat, but that seems erroneous. Modern knowledge of the mechanism of colour change should make it easy to clear up this question.

In contrast with the Amphibia the land reptiles, in particular the lizards, snakes and tortoises, are animals well known to live in dry environments. The probable reason for their ability to do so is that they are covered by thick horny integuments, usually scaly, which restrict evaporation of water. There is, however, some evaporation of water, though it is not known what proportion of it takes place from the lungs. The fact that skin temperatures of snakes are often above the mouth temperatures suggests that a high proportion of the evaporation occurs in the respiratory passages; the further fact that wind speed has rather little effect on water loss supports this suggestion (Benedict, 1932).

Benedict (1932) records total losses of water of the order of 0.1–0.3 % of the body weight per day at about 22° C. in fairly dry air for a 5 kg. *Python molurus*, a boa, and a 5 kg. tortoise, *Testudo denticulata*. These rates are to be compared with about 1 % per day for the mealworm, *Tenebrio molitor* (Buxton, 1930), about 4 % per day for the cockroach, *Blatta orientalis* (Gunn, 1933), and much higher values for the woodlouse, *Porcellio scaber* (Gunn, 1937) and the centipede, *Lithobius forficatus* (14 % per hour, Hawkins, 1939), under similar conditions. It is not easy to select a comparable figure for Amphibia, for in still air in a chamber the water lost changes the humidity considerably, while in a draught the rate varies enormously with the air speed and is not comparable with the results given above. The following seem to be the most suitable figures for comparison: 75 % of body weight per day for the frog (Adolph, 1932) and about 960 % per day (10 % in 15 min.) for the salamander, *Plethodon glutinosus* (Caldwell, 1925).

It is therefore to be expected that the body temperatures of reptiles will be relatively unaffected by evaporation of water. It has been shown by Benedict (1932), however, that for snakes under basal conditions in dry air the whole of the metabolic heat *and more* is carried off by evaporation. The result is that under these conditions—no movement and no digestion going on—the body temperature is slightly below air temperature (Fig. 4) and heat is gained from the surroundings by conduction. Benedict established this result not only by measuring body temperatures as well as he could and estimating water loss but also by measurements of exchanges of sensible heat in a compensation calorimeter.

The depression of body temperature of these large snakes in dry air is therefore clearly established, but it is nevertheless not possible to assign a representative set of values to the depression. In the first place it requires a number of men to handle a 5 m. python weighing 30 kg. when an attempt is to be made to take its oral and rectal temperatures. Handling raises the body temperature in two ways: metabolic heat is

increased because of the animal's struggles and heat is transmitted to the snake from the men's hands. Again, a large animal in air takes some time to reach a moderately steady temperature after any sort of change, and even then there are measurable and erratic differences between mouth and anus. Fig. 4 is as representative as any data available. In moist air or when the snake is moving, digesting or incubating its eggs, the body may be several degrees warmer than the air around it. When the skin is being shed water is lost more rapidly and body temperature falls as much as 3°C . (Benedict, 1932). Some data on smaller reptiles are given in Table 3.

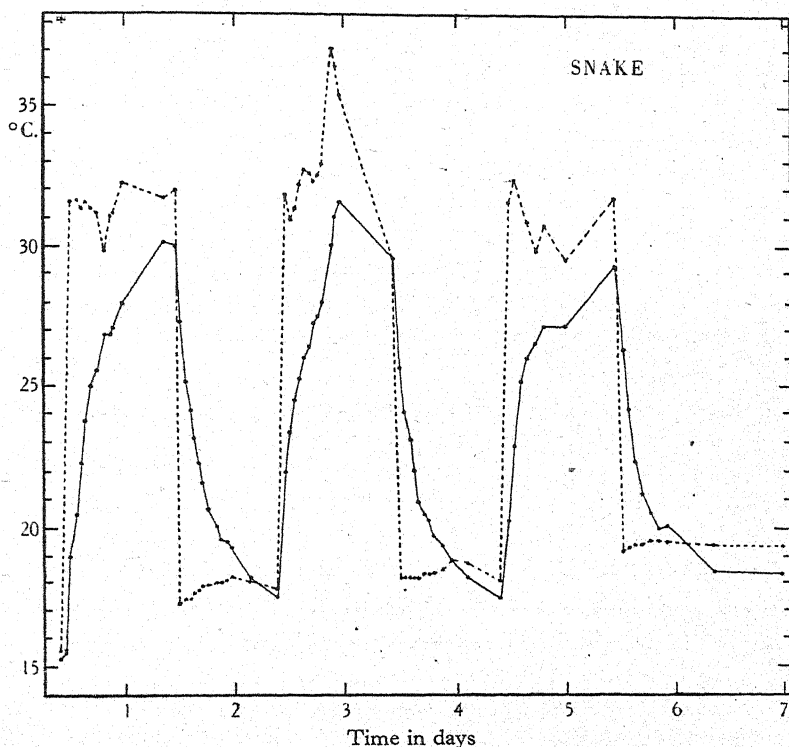


Fig. 4. The influence of changes in environmental temperature upon rectal temperature of a snake. The full line represents rectal temperature and the dotted line environmental temperature. To avoid the influence of handling, when body temperature was recorded the snake was not removed from its box but the tail alone was pulled out and the thermometer inserted in the rectum. (After Benedict, 1932.)

It has been suggested that owing to their large size and consequent relatively small surface available for heat loss the extinct giant reptiles may have had a blood temperature considerably above that of the environment. The analysis made by Benedict (1932) suggests that this is unlikely, if we are allowed to extrapolate from his data. Not only for reptiles but also for fishes and amphibians covering a weight range from 9 g. to 5 kg. the basal heat production per square metre of surface is remarkably constant; this implies of course that the large animals have a smaller heat production per kilogram than the small ones. If the same relation held for the giant reptiles and if the evaporation per square metre was also comparable to that of Benedict's snakes, then metabolic heat and latent heat of evaporation would be of the same order of magnitude and body temperature would not be elevated. It is possible of course that with a wider range of body

weights than it is possible to find to-day, reptiles would show an increasing heat production per square metre of surface with increasing weight as the mammals do (Benedict, 1938).

One result of the large size of these extinct reptiles is quite probable. Owing to the large heat capacity and small surface area, body temperature would follow changes of air temperature only sluggishly. Benedict (1932) found that when a large snake, a boa, was taken from 30.2 to 20.2°C., it required about 3 hr. for the body temperature to fall to within 1°C. of the new environmental temperature. Many small reptiles are inactive during the cool night and only become very active after warming up in the sunshine. Very large reptiles subject to the same limitation would take much longer to warm up under similar conditions and also, of course, much longer to cool down when the sun set. It seems likely, therefore, that the giant reptiles went to bed late at night and got up late in the morning.

In the evolution of homoiothermy, although the heat insulation provided by hair or feathers must have been important in maintaining a high body temperature, the essential advance shown by homoiotherms was the development of a high metabolic rate. Thus it has been shown by Benedict (1938) that, making due allowance for the accelerating effect of high body temperature, the rate of heat production per square metre of surface of homoiotherms is seldom less than five times as high as a representative value for reptiles.

VI. CONCLUSION

In analysing and assessing the factors determining body temperature in poikilotherms it is simplest both theoretically and experimentally to deal with the steady state, when the temperature is not changing. A balance-sheet can then be drawn up showing the relative effects of metabolic heat, evaporation, radiation and conduction. As far as any generalizations can be made on comparing animals which are fairly similar to one another we find that all these factors are related more or less approximately to the surface area of the body rather than to its weight. That is to say a mere difference of size would appear to have little effect on body temperature at the steady state.

On the other hand, when body temperature is changing, the surface-weight ratio is of great importance in determining the rate of change. The heat capacity of the body is not proportional to the surface but to the weight. If all the factors tending to alter temperature vary as the surface area, then a large animal will change in temperature more slowly than a smaller one. A large animal can, so to say, draw upon reserves of heat from its interior when its skin is being cooled and so delay the process of cooling.

In a similar way, as far as keeping cooler than the environment by evaporation is concerned, a large animal can maintain a given water loss per unit area longer than a small one. Large animals can afford to keep cool by evaporation; small ones cannot, for they dry up too quickly.

It seems then that at the steady state and when the variable of time is eliminated size is unimportant. When time comes into the equation and reserves of heat, water and perhaps food become important, then large size is valuable in resisting short spells of unfavourable conditions and the small animal is at a disadvantage.

Generalizations of this kind are both difficult to discover and unsuitable for application without qualification in particular cases. For example, in considering metabolic

heat, as far as possible the basic or standard metabolism—the lowest rate—has been used. Any activity on the part of the skeletal muscles or the digestive system leads to a higher heat production and the maximum *increase* possible is not the same in all animals; in some insects it is far larger than in man and so enables these insects to reach by muscular activity a body temperature comparable to that of homoiotherms. Considerable use has been made of the generalization that metabolic rates of animals are more nearly proportional to the surface areas than to their weights; but this generalization is not an accurate one and cannot be applied to widely different groups of animals. Again, the effects of radiation depend on colour as well as size. Where so little is known of details any guiding principle, however imperfect, is of value provided its limitations are not ignored.

VII. SUMMARY

Temperature, especially body temperature, is a very important factor in the lives of animals. Body temperature may be raised or lowered by the transfer of heat directly by conduction, or indirectly through the transformation of radiation into heat; it may also be raised by metabolism and lowered by the evaporation of water. These are the factors which determine the amount by which the body of an animal shall differ in temperature from the surroundings.

In aquatic animals, only conduction and metabolic heat are important and the body could not therefore remain cooler than the water. On the other hand, the water acts as a very effective cooling fluid, especially in animals with a good branchial flow of blood, and after a change of water temperature, body temperature becomes steady again in seconds or a few minutes. With one exception all recent measurements show body temperature to be less than 1°C . warmer than the water, even in a large cod. Temperature measurements are open to many errors, and the century-old belief that the tunny is warm-blooded requires confirmation.

Air, unlike water, is a good heat insulator. For land animals gains of heat through radiation and losses by evaporation are important; an environment at uniform constant temperature probably seldom or never occurs in nature and gradients of temperature in the animal body itself may be considerable.

Evaporation of water may cool an insect by several degrees below the air around it, especially if the air is dry and the temperature is as high as 40°C . There is no reason to believe that this cooling is an active regulatory mechanism, even in the special case of the cockroach, though it may save the insect from heat-stroke during a short exposure. The surface-volume ratio is larger for smaller animals, heat intake varies with the surface, and water available for evaporation varies with the weight. Consequently, other things being equal, if a small insect keeps cool for long it may desiccate too much. Even for a large insect prolonged exposure to warm dry conditions may be more damaging than a similar exposure to moist conditions, because the animal dies from desiccation.

Radiation, especially from the sun, is often an important factor which keeps insects' bodies warmer than the immediate surroundings, and differences of $5\text{--}15^{\circ}\text{C}$. have been recorded. The colour of an insect's cuticle is not a good indication of the degree to which it absorbs heating radiation.

The metabolic heat developed by similar insects at rest is very roughly proportional to the surface area of the body; since direct and indirect heat exchange and evaporation

of water are also related to surface area rather than to body weight, mere size of body should have little effect on body temperature under steady state conditions. In most comparisons of different species, of course, the constants to be applied to these factors will be dissimilar. Some insects can increase their metabolic rate enormously, and this power is used in elevating body temperature, especially before starting to fly. It seems unlikely that insects could maintain a high temperature by means of a coat of material of low heat conductivity, for with small objects such a coat actually increases the rate of heat loss because of the enlargement of the surface area. A heat balance-sheet shows that evaporation is the principal mode of loss of metabolic heat, even in rather moist air.

Amphibia appear to have no anatomical protection against losing body water and it is their behaviour which enables them to live on land. In dry air, especially in a current, evaporation is extraordinarily rapid, body temperature falls 5–10° C. or more, and death from desiccation occurs in a few hours. In saturated air the body is warmer than the air and some of the metabolic heat is still lost by evaporation.

In reptiles the skin is opaque to radiation and so protects the deeper layers of the animal. Like some insects lizards bask in the sun and the body may thus be warmed by as much as 20° C. The rate of temperature change is lower than in insects because of the larger size and the consequent smaller surface area per unit weight.

Evaporation from reptiles is slow, even slower than from xerophilous insects, if the rate of loss is expressed as a percentage of body weight. Even so a large snake when inactive is cooler than the surrounding air because of such evaporation as does occur. On the whole the heat relations of terrestrial reptiles seem to be similar to those of insects. There is no reason to expect a raised body temperature in large reptiles; the large size of some of the extinct reptiles probably had only the effect of making body temperature lag behind changes in the environmental temperature. It is the high metabolic rate of birds and mammals which enables them to maintain a high constant temperature, not primarily their coats of feathers or hair.

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THE NUMBER CONCEPTION IN ANIMAL PSYCHOLOGY

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Work on the problem of 'number conception' in animals was for many years discredited and obscured by controversy about 'clever dogs' and 'counting horses'. Their amazing achievements, as is well known, were finally shown to be the hardly less amazing fact that these animals had learned to act according to very minute movements of their owners or trainers. For the problem in question it matters little that the human part of these performing couples were either deceiving their audience on purpose, or were themselves deceived, not realizing at all that their animals had learned to react to hardly visible or audible signs, given unwillingly and unknowingly by their masters. It is worth remembering that even the learned committee set up to investigate the problem was also deceived. Shortly afterwards the real solution was discovered by Pfungst (1911).

I. THE FIRST SO-CALLED NUMBER TESTS

The first systematic experiments on the problem of number conception were carried out by Kinnaman (1902) and Porter (1904). Kinnaman used two rhesus monkeys (*Macaca mulatta*) as subjects and offered them a board 10 ft. in length on which 21 bottles or glasses of uniform shape were placed. These glasses were covered with white paper, so that the food in them could be seen only by looking into them from above. The animals had now to learn that food was only in one of the 21 glasses, at first in glass no. 4 from the right end. Later he tested the ability to find food in glasses nos. 2, 5, 1, and 3 out of a maximum of 11. Every choice was of course recorded, and a series of 30 tests was chosen as a unit. Apparently a maximum of 3 series = 90 tests was performed per day. It is not easy to judge Kinnaman's results properly as he gives only the record of tests carried out with glasses 1-6 but not with glasses 7-11, so there are some gaps, and it is obvious that every transition from one glass to the next influenced the choice of the animal. If we look at his tables we often see that a success achieved in one series disappears again

in the next. Thus when food was in glass no. 4 this glass was chosen 13 times in the 5th series (13 being the maximum of all choices of a single glass in this series), while in the next one the correct glass was chosen only 5 times, but glasses nos. 2 and 3 were selected 12 times each. In the case of other glasses (nos. 1, 2, 5, 6), however, the author obtained very satisfactory records. The choices of the correct glass increased in nearly every series and finally reached a definite maximum.

The author repeated his experiments with two children of 3 and 5 years, who had not been taught to count. Marbles were used instead of food. The older child learnt to locate the glasses nos. 1, 2 and 3, the younger one only nos. 1 and 2. As the rhesus monkeys were only about 1 year old a relative superiority of the monkeys compared with human beings was assumed by the author, but it must be remembered that a child of 1 year is in no way comparable with a monkey of the same age whose physical development is so much further advanced.

Kinnaman called his experiments 'number tests', but he himself expressed some doubt as to whether he really tested the number conception of the animals. 'Is it number, quantity, or form?' he asked himself. Strangely enough he omits the most probable explanation, namely, that the monkeys were trained to estimate a certain distance from the right end of the row of glasses. For if we look for control tests in order to exclude the effect of 'secondary cues' (marks on the glasses, etc.) we find that Kinnaman was careful enough to change the position of the board in the room and even changed the glasses, often 'after accuracy was established'. The distance of the glasses, however, was never varied. It would thus appear most probable that the position of the glasses decided the monkeys' choice. Even then compared with later experiments it is surprising that the animals were able to locate the glasses nos. 5 and 6 with considerable accuracy. But there is another source of error. The effect of marks on the glasses was excluded, but there might have been still some on the board, as apparently they were always placed on exactly the same spot on the board. This source of error should have been checked by using several boards, but Kinnaman apparently omitted to take this precaution. This might be the explanation of the unusually good results with respect to glasses 5 and 6 after a relatively short training (glass 5: 6 series = 180 tests; glass 6: 8 series = 240 tests to establish discrimination). Apart from this shortcoming we must give Kinnaman the credit of having first performed a well-devised set of experiments which in every respect are much superior to the contemporary work of Garner (1900, 1905) and others. Similar experiments were carried out by Porter (1904) with 'English sparrows' (*Passer domesticus*). He is already careful to speak of 'so-called number tests', but his method is, on the whole, a close imitation of Kinnaman's. Again a board with food glasses was used and the same precautions were taken to avoid marks on glasses (here only 6 were used), but here too the possibility of marks on the board was not excluded. A new feature in these experiments is that the orientation of the board remained the same, i.e. it was not turned through 180° when it was changed from one end of the cage to another. As a result of this glass no. 1 was sometimes at the right end of the row for the bird and sometimes on the left. Unfortunately, it is not clear from the records how often this change had been effected—a change obviously very important for the bird.

As the distance of the glasses was again unchanged, the same objection can be raised as in the case of Kinnaman's experiments. It is interesting that Porter allowed the birds to take one bit of food whether they chose the correct glass or not. 'The experiment of

making them do without when they did not alight on the right one was tried, but this only made them miss the more' (p. 330). Thus he realized the danger of 'discouragement' in such experiments. In addition, Porter found it important that his sparrows should not be tested immediately with another glass after they had learned to feed from one of them. The older association interferes with the new one unless a rest of some weeks intervenes.

The percentage of correct choices was in many cases greater than it was for Kinnaman's monkeys. Porter thought this could be explained by the fact that the birds were in smaller quarters (apparently absolutely, but not relatively). The explanation which appears more probable to me is that the sense of location or position is far better developed in birds than in most mammals (e.g. importance of finding the nest without the help of the sense of smell, etc.).

Porter himself is rather doubtful whether or not his experiments come 'any nearer to proving that animals actually count, or perceive the relations necessary for counting.... The method of experimentation does not require anything more than the location of the member of a series, or the sensing of the size of a group. But if we do not find in birds the power to count we have... something of that preliminary number sense which Ribot described as belonging to children and savages.'

Rouse (1906) tried to obtain a direct comparison of correct choices with regard to position, colour and form, but as the distance of the food containers was again unchanged his experiments do not show any advance in method.

II. THE MULTIPLE-CHOICE METHOD

We have to consider now a large group of experiments carried out chiefly by Yerkes and his collaborators, the so-called 'multiple-choice' tests. Here again a fixed number of objects (compartments or feeding boxes) is offered to the animal. This is to be trained to discover the box which contains food or the compartment which allows access to food. The fundamental innovation here is that, while the position of the boxes is unchanged, the position of the 'correct' box is always different. It is effected in the following way (Fig. 1): A certain number of boxes is presented in front of the animal which is set a 'problem', e.g. to select the second box from the right end. In the case of 12 boxes, all open, this would be box no. 11. In the next test only the boxes nos. 2, 3 and 4 are open and the correct solution is now box no. 3. In the following test the boxes nos. 5-9 are open and box no. 8 is now the correct choice, etc. In this way the correct box, although in a constant relation to the whole number of all open boxes, is always in a different place. In addition, the number of open boxes or open doors is always changing. This is of course a very important improvement compared with earlier methods, as all possible secondary cues on the boxes (visual, olfactory, kinaesthetic, etc.) are now excluded. By working out a system of different 'settings' (i.e. the total number of open doors in each test) and testing always identical 'problems' Yerkes hoped to discover the animals' degree of ability 'to dissociate the essential and constant relation of the right mechanism from its accidental and variable accompaniments', and to obtain comparable results as to 'abstraction' and 'ideation' of different individuals, different species, stages of growth (=different age), conditions of normality, etc. With these optimistic views Yerkes started his experiments (1914, human beings; 1915, in collaboration with Coburn, crows and pigs; 1916, monkeys and apes; 1927, gorilla; 1934, chimpanzees). The 'problems'

selected were originally: (1) the first box on the animals' left, (2) the second box from the right end, (3) alternately the right and left end box, and (4) the middle box. In the case of the last problem an odd number of boxes had to be offered, i.e. either 3, 5, 7 or 9 boxes, and for each problem 10 different 'settings' were fixed before the experiments started.

Early successes were obtained by Yerkes in experiments with pigs. They proved to be 'ideal subjects for studies in adaptive behaviour' and were able to solve the first three problems very well, while problem no. 4 (the middle box) was too difficult when more than 5 boxes (7 or 9) were used. (*Note.* A different interpretation of the solution to the 'middle' box problem is given below.) Yerkes found that the new multiple-choice method

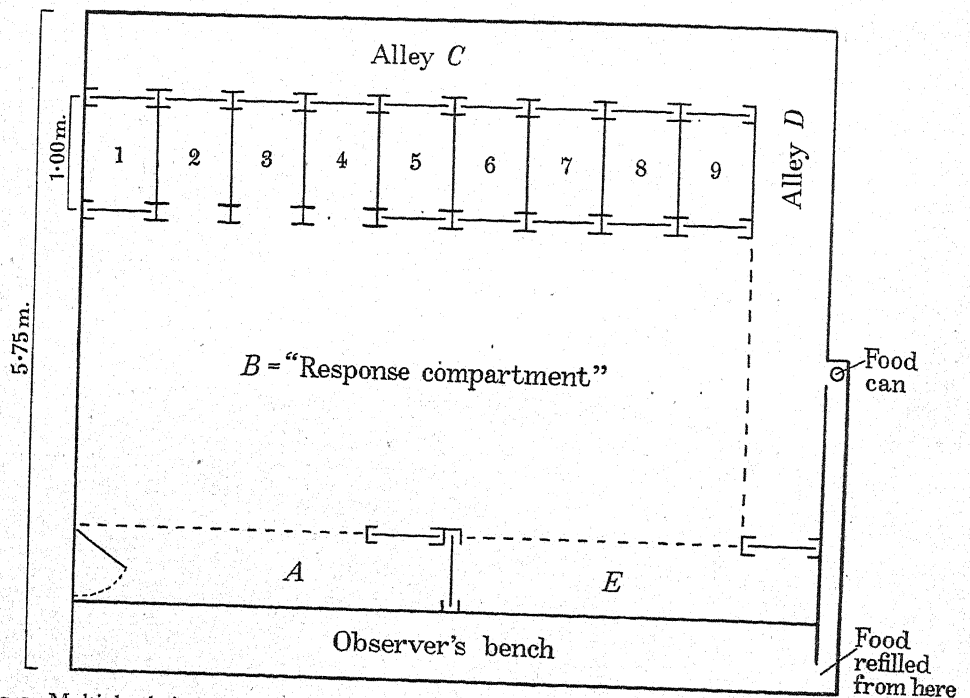


Fig. 1. Multiple-choice apparatus for chimpanzees, used by Yerkes. The animals start from compartment *A*, enter the 'response compartment' *B* and have there to choose the correct box which allows the passage to alleys *C* and *D*, where food is obtained. Then the animal returns through *E* to *A*. The sliding door between the entrance and exit doors of boxes 1-9, are operated by means of cords from the observer's bench. The boxes are open on the top and covered only with wire netting. This sketch shows the arrangement for control-setting no. 1: the entrance doors 2, 3 and 4 are open. The correct choice, the first open box from the left is therefore box 2. In the case of a correct choice the exit door is opened allowing access to food. In the case of a wrong choice the entrance door is closed and the animal detained in the box for a short time. (Slightly altered after Yerkes, 1934.)

had fully justified his expectation. But later experiments gave less satisfactory results, and gradually Yerkes's enthusiasm diminished. His last papers reveal a certain disappointment with the method.

There are, in my opinion, three different reasons for this comparative failure. First of all Yerkes has approached the problem with a preformed conception. He was anxious to find 'sudden' solutions indicating 'ideational' behaviour. The second reason is the rigidity of the method which has very rarely been adapted to the special conditions of

the problem, and of the animal. Last but not least the method of recording and judging the animals' progress was often unfortunate. For Yerkes tested his animals, during regular series, in every single 'setting' until they found the correct box, and there were often 3, 4, 5 or even 6 unsuccessful attempts before the right door was found. That means, however, that a series of 10 settings does not consist of 10 tests but actually sometimes of 20-40. On the other hand, all the errors made in a single setting are counted by Yerkes only as one. For example, in setting 5 of problem 1, first door on the left open (Yerkes, 1934, p. 16), the 5 doors 2, 3, 4, 5, 6 are open, 2 being the correct one, and the result in trials 51-60 (actually tests 114-135) is 4, 6, 6, 3, 6, 2. These 5 errors are recorded as only one. But in the next setting (no. 2) only the doors 8 and 9 are open, correct solution 8, and the result 9, 8 is again recorded as one error. In setting 8, open boxes 4, 5, 6, 7, 8, correct solution 4, the results 7, 4, or in another series the results 8, 5, 8, 5, 7, 4, are both equally regarded as one error. It is obvious that a proper judgement of progress is obscured in this way. Another difficulty is the method of judging the final effect of training. For Yerkes used quite arbitrarily as criterion for 'problem solution' a perfect series of 10 trials in succession. He says himself that 'this is a rigorous requirement, since it makes no allowance whatever for carelessness or distractions' (1934, p. 17). We know, however, that not only monkeys and apes but even human beings are often careless and distracted, and both of them become discouraged if one asks too much of them. Of course there is no objection to using the number of correct results obtained in succession as one of several indications of the progress of learning, but to make it the only one is certainly dangerous.

As to the rigidity of the method no attempt whatever was made during all the years to use new 'problems' or to make the distance of the boxes variable, or at least unequal. Only in one paper (1927), and here only in the case of one problem (the middle box), was the order of the open boxes arranged so that they were not necessarily adjacent, e.g. in addition to 1, 2, 3, or 3, 4, 5 the combination 2, 4, 5, or 1, 4, 5, etc., was used. This is of course a valuable improvement, but unfortunately it was soon abandoned.

Finally, the desire to see 'sudden' solutions by his subjects has no doubt influenced Yerkes's judgement (see below, Spence, 1939). When one of his chimpanzees after 160 trials with very varying results surprisingly produced 100% correct solutions during the next 10 tests (1934, pp. 30-2), Yerkes did not hesitate to consider this as a 'sudden success'. Every unbiased observer would surely first think of a 'secondary cue'. The fact that the ape was on this day especially eager to work confirms this opinion; and actually during the following trials the chimpanzee was totally unsuccessful. Thus the most plausible explanation of the previous 'success' seems to be that the experimenter prematurely tightened the rope used to open the correct exit door. The animal could therefore see the difference between this and the other cords through the wire-net ceiling of the box. This appears not improbable in the excitement of the observer's expectation, and there is no doubt whatever that animals can utilize such cues at once (cf. Bingham, 1922; Bierens de Haan, 1926).

Experiments carried out by Spence (1939) are to a certain extent a continuation of Yerkes's work. They were performed in the same laboratory, and some of the 17 chimpanzees used as subjects had been previously used by Yerkes. We must deal with this paper in detail, as it throws interesting sidelights on the possibilities and limits of the multiple-choice method.

Spence starts with a survey of the somewhat meagre results of previous researches and deplores the fact that the setting of the problem was not quite clear in them: 'Unfortunately, when simpler problems have been used, there has been considerable doubt as to whether their solution has involved a perception of spatial relationship' (p. 7). But Spence again did not alter the distance of the boxes and again used only boxes adjacent to one another, thus producing the same source of error. He made, however, two important alterations: he used a 'manual' type of multiple-choice apparatus (small boxes of $6.4 \times 7.6 \times 7.6$ cm. instead of the large compartments through which the animals had to pass¹) which was certainly an advantage as the apes could now better see the relation of the correct box to the others. Secondly, he kept the number of boxes constant in the various settings, which is a dangerous simplification of the procedure, and further only the boxes used in the setting were visible to the animal, while the rest were withdrawn completely. For the first problem (the middle-box in Spence's investigations) never more than 5 boxes were used. (Concerning the risks of using such a small number, see below.) Problem 2 was the second from the left of 6 boxes, problems 3 and 4 the right and left end-boxes out of 7, and problem 5 the relearning of problem 1. No alternation tests were performed. This limited choice is all the more regrettable as Spence himself admits apparently that the two end-box problems do not involve 'ideation' at all.

Another simplification of the method is that Spence did not use 10 different settings, but only 4 settings given 3 times each in irregular order. That means in learning series A of problem 1 the correct box (middle one of 5 boxes) was always 3, 5, 7 or 9 as the settings were 1-5, 3-7, 5-9, or 7-11, the order for the correct box in the 12 settings being 7, 3, 5, 9, 7, 5, 9, 3, 5, 7, 3, 9. In another series, B, the correct boxes were always 4, 6, 8 or 9, when the settings were boxes 2-6, 4-8, etc.

The 'rigid' criterion for problem solution used by Yerkes was kept by Spence, and he demanded even more, as the learning trials were continued until the subject responded without error in all 12 trials (Yerkes used only 10) of a series. This meant sometimes 1000 or even 1400 trials for one ape. Although 15 out of 17 chimpanzees were eventually able to satisfy this 'rigid' principle, control tests carried out subsequently show what little value can be attached to such a method of judgement. Only 1 of the 15 subjects then produced a 100% correct solution, 4 between 85 and 80%, 1 obtained 55% and the remaining 9 chimpanzees only 50-0%. Even more significant is the fact that in control tests with 7 boxes (box 4 now being the correct one) not a single animal responded with 100% success, the best solution being now 70%.

Spence summarizes his experience with the 'middle of 7 boxes choice' in the following way (p. 24): 'While there is a tendency for the middle (fourth) box to be chosen most often by the subjects that scored high in the regular control tests, it will be seen that the correlation is by no means perfect. The difference in the scores of the two groups (more or less gifted ones) is much less in the 7 box tests than in the previous 5 box test, for the poorer group of subjects did about as well on both tests, whereas the subjects that scored high in the 5 box tests fell down somewhat in the 7 box test.' While there is hardly any doubt that some of the apes had really grasped the 'idea of middleness' (one specimen was even used for a test with 9 boxes and chose the middle box in 60%; she had chosen the middle box of 7 in 70%, and middle box of 5 in 100%) there might be for the

¹ Small boxes had been used previously by Sadovinkova (1923) (see below), but as the subjects were here small finches they are in effect equal to the large compartments used before.

'less gifted' chimpanzees another possibility, not considered by Spence. There is the chance that the ape, in the case of 5 boxes, did not actually choose the middle box but learned only to avoid the first and second one from one end, or in other words to choose the third one from one end. This seems to me very probable at least in the case of the chimpanzee Wendy which had 80% correct solutions in the 5 box control tests, but chose in 20 control tests with 7 boxes the correct box (no. 4) only seven times, no. 3 eleven times and no. 5 twice. This idea is corroborated by experiments with monkeys in which Ohtsuka (1939) found that the animals were able to find the middle of 3, but not of 5 holes, and control tests showed that the animals had actually trained themselves to choose the second hole either from the right or left end.

One very interesting result of Spence's experimental data shows how fortunate he was in using so many animals. While there is no indication of any age differences in the capacity of 'problem' solving, there was, at least in the middle-box problem, clear evidence that one group learned the specific settings while another group responded to some characteristic common to each setting. Two graphs (separate learning curves of the four different settings of problem 1) reveal this in a striking way. The four curves of a gifted chimpanzee drop below the chance limit at about the same time, while in the case of a less gifted one at first one curve drops down, then a second one, and so on.

This fact appears to me a much more convincing proof for an understanding of the 'general idea' of the problem than Yerkes's 'suddenness of discovery'. Spence himself remarks rightly that very often sudden shifts occur from one wrong response to another wrong one, and even from a right to a wrong response, 'but the insight writers seem to recognize this criterion (the sudden change) in the case of correct or adaptive responses, but ignore it in non-adaptive responses' (Spence, 1939, p. 49).

Before we conclude this section we have to consider one more paper dealing with multiple-choice, this time with small birds, by Sadovinkova (1923). It is important, as the author claims that her finches finally obtained 100% positive results in selecting the middle-box out of 9, a success never achieved before or after her experiments. It is therefore understandable that her results have been widely quoted in all papers dealing with multiple-choice and similar problems. Actually the results are even more startling, as these birds were able to master two or three different tasks simultaneously (one of the most difficult problems for animals and children) in response to various 'indicators'. For example, of a number of boxes varying from 2 to 9 the first from the left had to be selected if no paper was present (A), the first from the right end when a white paper was present (B), and the middle-box when a black ribbon was stretched across the area (C). One of the birds, a little serin or wild canary (*Serinus canarius*), learned all this in a few days. The author is convinced that here a real case of 'ideational behaviour' (in the sense of Yerkes's) had been displayed and that the bird had grasped 'the fundamental elements of counting'. This is of course untenable, as it is quite possible to choose one end-box, or the second box from the end, without counting. But the results are nevertheless surprising. If we now look at the experimental procedure it seems at first that Sadovinkova was even more careful than Yerkes in avoiding any 'clever Hans errors' as she improved the experimental procedure considerably by making the doors to the boxes work automatically. The boxes were constructed like a trap cage, and their entrance doors slid vertically down as soon as the bird entered the box. Of the exit doors only the 'correct' one was open, so the bird was trapped in the box in the case of an incorrect choice. The

observer left the room and returned only when she heard the sound of one of the falling entrance doors. She then knew that the choice had been made.

Unfortunately, there can be hardly any doubt that, in spite of all these precautions, Sadovinkova has been 'outwitted' by her birds. For the exit doors were swinging doors and the 'wrong' ones were locked by means of a pin let down from above. There is no indication whether these pins were outside or inside the box, but if they were inside they were certainly visible to the bird sitting in front of the open entrance door, and even when they were outside they must have been visible, too, as these exit doors consisted of perforated zinc. The fact that her Fig. 2 illustrating the mechanism is reproduced by mistake in a wrong position (turned 90°) is perhaps the reason that this has not been found out long ago. Even the monographs by Fischel (1938) and Bierens de Haan (1940) still report Sadovinkova's achievements as 'successes' without any doubt in the experimental technique. Probably the bird learned only to find out which exit door was unlocked before it entered any box, and there is no report on control tests in which several or all exit doors were unlocked. If the bird used such a simple secondary cue, however, then it is not surprising that it solved during the last 2 days all of the 21 very complicated tests without any error, and the learning of three different tasks simultaneously was not necessary.

III. THE TEMPORAL MAZE AND THE ALTERNATION PROBLEM

We come now to a group of papers which attempt to solve the problem of number conception in a different way, namely, by examining the ability of animals to perform actions alternately, either in simple or multiple alternation. Strictly speaking the 'alternation tests' of the early multiple-choice papers belong to this group as well, and we may recall that a simple alternation (right and left end box) was achieved only by few animals (pig, Yerkes & Coburn, 1915; rhesus monkey, Yerkes, 1916; chimpanzee, Yerkes, 1934) while others were unable to solve this problem (crow, Coburn & Yerkes, 1915; rat, Burr, 1916).

Hunter (1920, 1928) was the first to deal with this problem systematically. He used the 'temporal maze' which is entirely different from the numerous forms of the well-known ordinary or 'spatial' maze. The temporal maze is not in fact a maze, but it becomes one temporarily by the opening or shutting of doors. For example, in the following scheme (Fig. 2) the subjects have to run first in the direction *ABCA* and the second time *ABDA*. The training is effected by the opening or shutting of 4 doors (indicated by dotted lines) in turn, and in addition by punishment with electric shocks in the case of a wrong choice. In the decisive tests, all the doors were open and no punishment was given. One important point in the 'theory' of the temporal maze is the fact that even in the case of a simple alternation (*ABCABDA* or *rl* = run to right followed by run to left) the alley *AB* is common to both runs, the first and second one; and as Hunter points out 'differential sensory factors cannot be located therein, not only because it is common to both right and left responses, but because it is also constant and unchanging in its role of a common factor throughout the test' (1928, p. 376). And he adds: 'It is impossible for one and the same stimulus to cause first one response and then another unless it is supplemented by some factor either inside or outside the organism.' This is an overstatement in the case of a simple alternation (*rlrl*) because the alley *AB* is entered from different points right and left from *A*. In the case of a double alternation, however (*rrll* or *rlrlrl*), the

statement is correct, *AB* being approached from the same side in the case of the second *r* (second turn) and the first *l* (third turn). This consideration illustrates the difficulty of the double alternation problem. Carr (1917) and Hunter (1920) found that white rats were only able to solve the simple but not the double alternation. Racoons, on the other hand, showed so much 'ability' in Hunter's well-known delayed reaction tests that he felt justified in assuming that this animal 'possesses the bare rudiments of "symbolic processes"' and should therefore be able to learn the double alternation temporal maze as well' (1928, p. 378).

The interesting result was that three out of four racoons (*Procyon lotor*) learned the double alternation *rrll*, but not a single specimen was able to extend this to *rrllrrll*. Exactly the same results were obtained by Gellerman (1931) working with monkeys and Karn (1938) with male domestic cats. The latter still claims that 'the double alternation

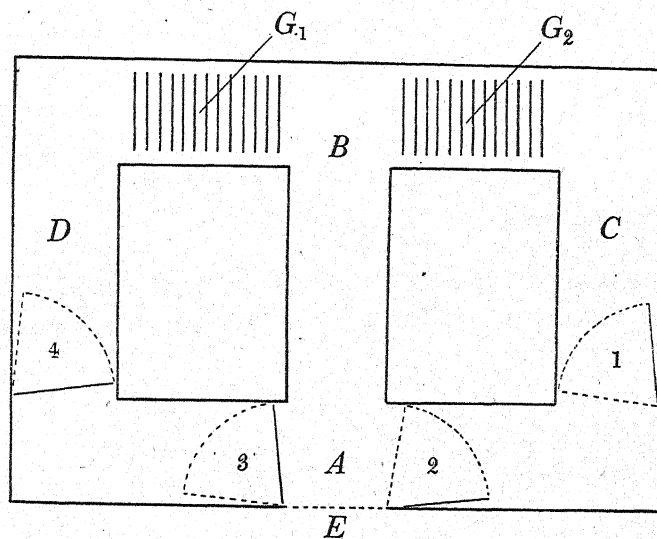


Fig. 2. Ground plan of the temporal maze used by Hunter (1928). *E*, entrance door; 1, 2, 3, 4, hinged doors; *G*₁ and *G*₂, electric grids. (Slightly altered from the original.)

technique is an ideal one for making comparative tests in that it can be used in essentially the same form with animals ranging from the lowest to the highest phyla'. Unfortunately one of the two cats (only two were used) after a good start refused later to work: it had to be forced into the apparatus, scratched and clawed at the entrance door, urinated in the apparatus and the experimenter came to the conclusion 'that the collapse of the animal's differentiating ability would seem to be best described as a case of experimentally induced neurosis'. The promised complete report on this neurosis has apparently never been published, and I therefore assume that this animal was probably on heat for the first time, the symptoms described being quite characteristic of a cat in this condition kept in solitary confinement.

The problem of alternation had already been approached earlier by Katz & Révész (1909) by means of a simple but instructive method. These authors, working with hens, used rows of grains of which every second one was glued to cardboard, and the birds learnt quickly to eat only the loose ones without touching the fixed ones. According to the authors' report one hen learnt also to select every third grain without touching the

grains 1, 2, 4, 5, 7, 8, etc. No hen was able to select every fourth grain. But even the selection of every third grain is a surprising achievement, much more so than the double alternation of Hunter's racoons. Révész (1922*a*) extended these claims. He stated that after completing the training the distance of the grains could be doubled (or halved) without destroying the 'alternation habit'. Unfortunately, a closer survey of this paper reveals a complete lack of any report on control tests in which all grains were loose. This is all the more surprising as Révész himself stresses the necessity of such tests, and their results would be highly illuminating. One is therefore forced to the conclusion that the birds learned only to distinguish between fixed and loose grains. One bird was, according to Révész, even able to alternate in an irregular way, e.g. instead of eating the grains nos. 2, 4, 6, 8, 10 it pecked 2, 8, 4, 10, 6. This is considered by Révész as a disproof of the 'hypothesis of innervation' (to a certain constant pecking distance), but as again no control tests are mentioned it only confirms my opinion that the bird was guided solely by its ability to discriminate between fixed and loose grains. The reported failure of the birds to select every fourth grain is of course not explained in this way, but as there are hardly any details given of the experimental procedure, it is impossible to find an explanation of this discrepancy.

In my view even a simple alternation, performed in a 'multiple way' without any secondary cues (discrimination of fixed and loose grains) and independent of the distances between them, can be considered as a preliminary stage to counting. An enormous gap, however, remains between this and counting in the 'human' sense of the word. I have checked the problem (Honigmann, 1942*a*) and obtained the same result when using the same methods as previous workers, but as soon as all secondary cues were excluded this easily obtainable training effect disappeared. It was, however, possible to achieve a true alternation in rows of grains all of which were loose, but it now took 500-600 training experiments (17-20 training days) to obtain this result, which was still by no means 100% faultless. On the contrary, it was most interesting to see how easily the alternation was abandoned as soon as any detail of the experimental data was changed. The statement made by Révész (1922*a*) that the distance of the grains does not matter at all—that it could for instance be doubled—was not confirmed. In such cases the whole row was eaten at once, but alternation took place immediately afterwards when the standard row was presented again. This, however, does not at all confirm the opinion that here a training to a fixed distance is demonstrated, for an alteration of the distance did not matter when it was introduced gradually. Even in rows of grains with unequal distances an alternation was kept when the distances increased (or decreased) gradually, and then indeed the distance apart of the grains at one end of the row could be half the distance at the other end, or double the distance. On the other hand, a standard row having equal distances was completely eaten (odd and even grains) when presented far from the usual place or on a base of different colour. The whole problem is in fact much more complicated than was previously assumed. It may surprise many readers that such 'irrelevant' items can influence the animals in a decisive way, the essential conditions remaining unchanged, but similar observations have been made again and again (Köhler, 1918, p. 12).

IV. 'GENUINE NUMBER CONCEPTION' SHOWN TO BE A TRAINING TO A CERTAIN RHYTHM

A genuine number conception in animals was assumed by Gallis (1932) and Giltay (1933). Gallis trained a bonnet monkey (*Macaca radiata*) to open the experimenter's clenched fist twice; each time the monkey obtained a mealworm hidden in the closed hand of the observer. The third time his fist was empty and the monkey learnt to open the hand twice, but not again. Later it learned in a similar way to obtain 3 mealworms by opening the hand thrice, but not for the fourth time. When this was achieved the first experiment was altered so that the monkey sometimes found 2 mealworms when it opened the hand for the first time. This was done in order to see whether the monkey would refrain from opening the hand a second time, which would prove that it understood that $1 + 1 = 2$. The corresponding modification of the second experiment using 3 mealworms was that the hand of the experimenter sometimes contained 2 mealworms the first time. If it now refrained from opening the hand after having obtained 3 mealworms this would prove that the animal understood that $1 + 2 = 2 + 1 = 3$. As a result of his tests Gallis concluded that his monkey had indeed the conception of 2 and 3. Giltay (1933) repeated these experiments with fowls and found that her hens were able to grasp the conception of 2, but not of 3.

Bierens de Haan (1935) repeated Gallis's experiments with the same result. He again trained a monkey (*Macaca irus*) to open his hand twice and to leave his hand untouched for the third time by offering him $1 + 1 + 0$ pieces of banana. The fruit was taken by the experimenter out of a dish which was of course not visible to the monkey. On the eighth training day the monkey worked correctly. But now Bierens de Haan interposed a very necessary control test—neglected by Gallis and Giltay—by offering $1 + 1 + 1$ pieces of fruit. The result was that the monkey at once opened the hand for a third time, thus proving that he was able to see whether the experimenter's hand was empty or not.¹ The experiments were now modified: instead of the hand a tin was used which covered the pieces of fruit lying on a table, and—as it was expected—the training was completely lost. We cannot here deal with these highly interesting and carefully designed experiments in detail, but we must consider the result. The author came to the conclusion that the monkeys learned nothing more than a certain rhythm of action, and control tests confirmed his opinion. He extended the interval between the 3 single tests (+ + -) from 10 sec. to 20 and 30 sec., with the result that errors increased as shown in the following record:

10 sec. interval (standard)	10 tests	0 mistakes
20 sec. interval	10 tests	3 mistakes
30 sec. interval	10 tests	9 mistakes

¹ The same was obviously the case in the experiments of Gallis and Giltay which are not convincing at all owing to the absence of control tests. Nevertheless, Spence (1937) in a review reports simply Gallis's results 'which led him to conclude that his subjects understood the concept of the numbers 2 and 3' without mentioning Bieren de Haan's just criticism, although Bieren de Haan's paper is mentioned in the same review. Lashley (1940), while acknowledging the unusual ingenuity in devising successive series of experiments, has again very severely but justly criticized the complete lack of adequate controls in the papers of Verlaine (1938a, b, 1939) and his collaborators Gallis (1932) and Tellier (1935).

A simple alternation (+ -) was much easier to learn and was not so easily destroyed by extension of the intervals as the previous rhythm, e.g.

10 sec. interval (standard)	10 tests	0 mistakes
20 sec. interval	10 tests	1 mistake
30 sec. interval	10 tests	6 mistakes
45 sec. interval	10 tests	10 mistakes

Another interesting by-product of these experiments is the result that the rhythm + + - started for the animal only with a successful test. That is, the monkey kept on lifting the tin covering the fruit until his quest was successful for the first time. He then lifted the tin for a second time again obtaining food, but the third time refrained automatically (compare Honigmann, 1942 a).

Bierens de Haan¹ has dealt in two more papers (1936 a, b) with the problem, and there is no doubt that his criticism is justified and that in these experiments there is no question of real counting involving the concept of number.¹ On the other hand, it is possible to overcome the mere training to a rhythm and to train birds to perform the same action several times in succession independently of any temporal rhythm (see below the papers by O. Koehler and his collaborators).

In contrast to the papers by Gallis and Giltay the training of birds to 'rhythmical motor functions' was the primary aim in a paper by Schole (1934). He used a new and original experimental technique (Fig. 3): his fowls were placed in a narrow cage the bottom of

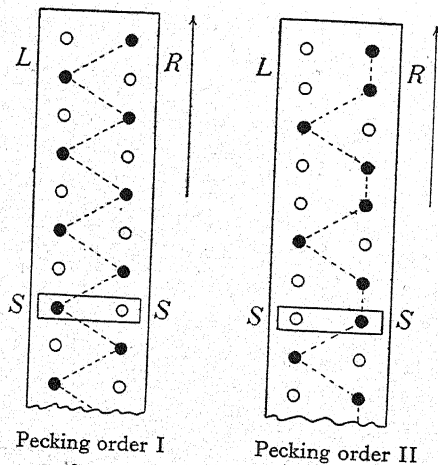


Fig. 3. Sketch showing front parts of movable boards used by Schole (1934). The black circles indicate holes filled with grains according to 'Pecking order I' and 'Pecking order II'. The dotted line shows the sequence of pecking, but it must be realized that the actual pecking movements of the bird were a change from right to left as only two holes were visible at a time through the stationary slot S \square S. (This sketch is not in the original paper.)

which (50 x 18 cm.) had a transverse slot of 16 mm. width. Underneath this bottom a board of 100 x 11 cm. could be moved. This board had on each side 50 holes of 15 mm. diameter and 4 mm. depth, the distance between the rows being 75 mm. Thus the whole

¹ Bregadze (1937) has tried to determine 'whether a dog could count 4' by combining a sound 4 times with food and 4 times without reward. After 91 days the dog (apparently only one was used) refrained from approaching the food box at the fifth sound and the habit was considered to be established; it was unstable, however. I have been unable to obtain the original paper, and from the review in *Psychological Abstracts* it seems that the dog started 'counting' only from the first successful trial (= food received). Possibly the training was again only to a certain rhythm, as no control tests with extended or reduced intervals are mentioned.

board had 100 holes. It was not moved at a uniform speed, but in jerky movements by hand, so that only two holes at a time were visible to the birds through the slot. The birds were then trained to eat grain out of these holes in four different 'pecking orders', e.g. pecking order I was a simple alternation from right to left and in pecking order II there were 2 grains on the right and the third one on the left, etc. The author's idea was apparently to find out whether the various kinds of rhythmical movements could be kept by the birds when either additional grains were offered or even all holes were filled, or, on the other hand, when holes were empty in contrast to the arrangement in the previous training. This is an interesting and new idea, but unfortunately the author's execution of his experiments is rather inadequate, and his control tests so insufficient that it is impossible to draw any safe conclusions from them. The author's claim that his results make 'the temptation to assume real counting in the fowl even greater than in the case of grains offered simultaneously' (viz. the experiments by Katz and Révész) is not justified at all. Actually his experiments prove hardly anything, but if repeated with a better technique and perhaps with the help of cinematography they should yield interesting results.

V. THE DISCRIMINATION METHOD AS AN APPROACH TO NUMBER CONCEPTION

(1) *The possibility of misinterpretation of results*

Before we start with the last group of papers dealing with number conception in animals we have to consider some dangers involved in the so-called discrimination method. We cannot deal here with the problem of how discrimination is actually achieved by the animal. A spontaneous choice is to be expected only if one of the objects in question has a primary 'valence' (to use a term introduced by Russell, 1934) for the animal, e.g. favourite food compared with disliked food, or simply more food compared with less food. But if the experimenter wishes to find out whether one animal is able to discriminate between, say, triangles and squares of identical area, colour, brightness, etc., secondary valences will have to be introduced as the characters mentioned have obviously no adequate biological meaning for the animal. The usual way is to confront the animal in a 'discrimination box' with a pair of stimuli and to reward the 'correct' and/or to punish the 'wrong' choice. It was soon found that the 'discrimination habit' can be learned in two different ways. The learning effect may be fixed either to one of the stimuli, or, on the other hand, to the relation between the two stimuli employed for the training. For example, if the choice is between two different shades of grey *A* and *B* and the animal has been successfully trained to select the darker shade *B*, it may in a new experiment select a third shade *C* (darker than *B*) when this is presented together with *B*. ('Structural function of the pair', according to W. Köhler, 1915, 1918.) This possibility must be considered in each separate case.

There is, however, another danger in this apparently simple method, a danger which has hardly been realized before and has often given cause to wrong interpretations of the animal's behaviour. This is the fact that the experimenter never knows, in the case of a non-relational choice, which of the two 'stimuli' is decisive for the animal. It is possible even in the case of training without any punishment that the animal learns only to avoid the negative stimulus, although this is not the 'emphatic' one here, or to select the positive stimulus although there is no reward in this case. Actually non-punishment is a reward and non-reward a 'disappointment'. The important point is that in such cases only one

stimulus obtains a (+ or -) secondary valence, and the animal can remain completely indifferent to the second stimulus. The neglect of this possibility, easily checked in control tests, has given rise to many misinterpretations.

Breed (1911) has already shown how differently animals can react in this way, but as he was apparently somewhat unfortunate in expressing his results his paper has not found the deserved recognition (cf. Honigmann, 1942*b*).

(2) *Discrimination between numbers of successive stimuli*

We begin this section with three papers in which two different acoustic sequences are used to train animals to discriminate between 'numbers'. Kuroda (1931) trained a monkey (*Macaca irus*) (after some preliminary experiments which have no relevance to our problem) to discriminate between one and two strokes of a bell. The monkey had to take food out of a hole designated as no. 1 when it heard one stroke of a bell, and to take it out of hole no. 2 when it heard two strokes. This was achieved after about 700 trials. But when a third hole was used, and the attempt was made to associate this with three sounds, the whole training was upset and, according to the author, 'all the past learning forgotten. For instance, the animal tried to reach hole no. 3 when it heard one stroke of the bell, and vice versa'—which apparently means that on hearing three strokes it reached for hole no. 1. Here again it is possible that the monkey had not suddenly forgotten his training, as Kuroda assumed, but that he had trained himself solely to avoid hole no. 2 when he heard one sound only. This would explain that he chose hole no. 1 or 3 without preference when he heard one stroke. It is even more probable that only the two strokes had gained significance for the animal (namely to choose hole no. 2), for this explains his subsequent behaviour even more fully: he chose holes nos. 1 and 3 when he heard one stroke or three strokes. It is therefore not necessary at all to assume a 'retroactive inhibition' as Kuroda does. Other weak points of this paper are the facts that only one specimen was used and that the 'completed training' was observed only for one single day—then a new training was begun at once.

It is interesting to compare this paper with the results obtained by Woodrow (1929). This author trained three rhesus monkeys to a definite relational choice (or response to a complex). The animals had to reach for food in response to whichever of two stimulus series contained the greater number of sounds. One monkey learned to distinguish 1 from 3, 2 from 3, 3 from 4 and—with a low degree of reliability—even 4 from 5. It failed to discriminate between 5 and 6. Another monkey achieved the same result but could not discriminate between 4 and 5 and 5 and 6. A third one learnt only the difference between 2 and 3, but this specimen turned out to be seriously ill and died 3 months later. The two other monkeys showed 'transference' of their previous training, i.e. when changing from 2:3 (3 being +) to 3:4 (3 now - and 4 +) the curve of learning showed a high start and a further rapid rise. Later, however, the curve dropped again, and the author says quite rightly that it is impossible to determine whether this retrogression is due to the loss of specific beneficial effects of previous training, or to the loss of interest or motivation, or both.

The most interesting fact is perhaps the observation that a change of quality of the sound (not now produced by knocking the food can as before, but by an electro-magnetic sound-hammer) 'destroyed' the training: the monkey was almost completely 'thrown off' and had to relearn the discrimination. This shows again how differently psychologists

and animals react. The training was certainly not destroyed, but for the animal the decisive factor was not the sound itself but the individual sound of the food can.

The discrimination, however, was not based on the 'abstract property' of numbers, the '2-ness' or '3-ness'. For when the interval between the sounds in the group of 2 was made equal to that between the first and last sounds in the group of 3, so that the total time required for each stimulus series was the same, the monkey had to be trained afresh.

Similar results were shown in a second paper by Kuroda (1937).¹ It was difficult for rats to discriminate 1 from 2 and 3 acoustic stimuli, but the animals were able to differentiate 5 from 1, 2 and even 3. Some individuals succeeded even in the differentiation between 5 and 4 sounds.

(3) *Discrimination between two quantities of objects offered simultaneously*

We come now to a group of papers dealing with the discrimination between numbers, or better quantities, of objects presented simultaneously to animals. It is of course necessary in all these experiments to change the position—right and left—of the 'correct' stimulus continuously but irregularly—otherwise a self-training of the animals to one side takes place very quickly ('side-constancy').

Révész (1922*b*) had already shown that fowls, spontaneously preferring a larger amount of grain to a smaller one, are able to discriminate between 2 and 3, 3 and 4, 4 and 5, 6 and 8, 7 and 10 grains. Beritov & Akhmeteli² (1937) trained pigeons to eat from the smaller of two piles of grain, viz. to select a pile of 3 grains instead of 6. The whole problem, however, was first dealt with in a systematic manner by O. Koehler and his collaborators who spent years of intensive work in well-devised and carefully controlled experiments. The work was begun by Fischel (1926), who used various kinds of birds. He obtained the best results with a goldfinch (*Carduelis carduelis*). This bird learnt by means of the gluing method to discriminate 3:1, 3:2, 4:2, 5:3, 6:3, but not 5:4, 7:5, 10:6 grains of hempseed, although 5:4 was practised in more than 300 tests. After this failure the discrimination 5:3 was forgotten and fresh training was necessary. The same applies to the pairs 6:3 and 8:4. A garden warbler (*Sylvia borin*) was able to take only one ant pupa from a heap, but could not be trained to take 2 and to leave the rest. Some pigeons (*Columba livia* dom.) distinguished . from .. and even .. from ∴ fairly well on the cover of food boxes, but when the 3 grains (or painted dots) were in one line ... no discrimination was possible. The birds learned much more easily and quickly the difference between Δ and | than the discrimination of numbers of grains or dots. We find again the fact that in a new position the characters used previously lost their meaning (their secondary valence) for the animals, and new training was necessary.

Fischel's training 'record' has been broken by Koehler, Müller & Wachholtz (1935) and Koehler & Wachholtz (1936) in experiments with pigeons. There are several reasons for their better results. Fischel found only 5 out of 9 birds 'suitable'. Müller, who had bred pigeons as a hobby for many years, reared the subjects for the psychological experiments by hand and obtained in this way extremely tame and suitable birds. But much more important is the fact that in the following experiments the ratio of reward and punishment was much better balanced. An extensive control by filming the procedure proved to be a very valuable help in judging the progress of the birds. A new device for

¹ I was unable to obtain the original paper and could only read a review by the author in *Psychol. Abstr.*

² The original paper in Russian was not available; quoted from *Psychol. Abstr.*

punishment was the 'frightening spring-board' which suddenly threw the cardboard with the grains upwards in the case of a wrong choice, thus frightening the bird extremely. As this device worked, with the help of springs, levers and bolts, according to the 'all-or-none law', the pigeon received no previous warning, and the observer was invisible and inaudible. It would have been a great mistake, however, to start the experiments in this way. The birds were at first gently driven away by the hand of the experimenter in the case of a wrong choice. But later the device proved to be very valuable and was made even more frightening for obstinate sinners by using compressed air or even a jet of water at the same time.

With the help of this punishment the discrimination between 5 : 4, 6 : 4, and even 6 : 5 was made possible, but there were occasionally up to 41 % mistakes. New training was necessary for every group of numbers, thus proving that the pigeons did not simply eat the larger amount of two heaps of grain. One pigeon learned after a great deal of training to repeat an action a fixed number of times. The limit of attainment, when offered a varying number of grains, was 6. Koehler calls this 'basic ability no. 2' in contrast to 'basic ability no. 1', which means exercise of the choice between different numbers, e.g. between 2 and 3 or 4 and 5 grains. Other interesting observations are that it was easy to train the birds to small differences in the size of figures, but difficult to train them to different geometrical forms. On the other hand \therefore against was possible, but when the 4 grains were grouped \therefore against ... the birds chose according to form (triangle) rather than to the number. Some discriminations of 'numbers', which were at first impossible for the birds, could be enforced by starting from a training to form and subsequent wiping out of the 'figural help' (compare Arndt's paper below).

The second paper (1936) is an elaborate attempt to eliminate figural and positional cues. It is illustrated by many double pictures of the grouping of the two amounts of grain between which the pigeon had to choose. Nearly 10,000 new tests were performed with one pigeon and the results very clearly recorded. 'Spontaneous tests' (i.e. without punishing device) often led the birds to abandon their previous training. These, however, were necessary in order to gauge the progress of learning, and it was definitely a mistake to perform too few of them in their earlier paper (1935). Only 50 such tests were performed all of which were filmed:

Trained to eat x grains	How many tests	Number of grains offered	How often eaten										
			3	4	5	6	7	8	9	10	11	12	13 grains
$x=3$	4	4-7	3	1	—	—	times						
$x=4$	8	5-9	1	3	4	—	—		times				
$x=5$	14	6-11	—	1	8	4	1	—	—	times			
$x=6$	24	7-13	—	3	6	10	4	—	1	—	—	times	
Total	50											times	

(Slightly altered from the original.)

In the last series ($x=6$) there are only 10 tests positive and 14 negative, and the learning curve showed no permanent rise. It was therefore not thought advisable to try the training $x=7$.

The question as to whether the number of grains offered influenced the frequency of positive tests was examined as well. For example, it was all the more difficult to stop the

bird eating while there was still present a large quantity of grain. The question of the influence of a possible pecking rhythm was also investigated. The fact that the pigeon often pecked several times at one grain, but nevertheless ate the correct amount, induced Koehler to deny such an influence 'as it was more difficult to believe in a swallowing rhythm than in a pecking rhythm'. This is, of course, no convincing argument, but Koehler's collaborators later excluded the possibility by means of new and reliable methods.

Arndt (1939) continued the work and improved the technique by various new devices. Most important is the use of a turntable (or a chute) which enabled him to offer peas to the pigeons in a varying rhythm (see below). Extension of the rhythm, i.e. increase of the intervals, by means of a moving glass pane gradually uncovering the peas frightened the birds too much. In addition Arndt continued on the lines of Fischel's experiments by offering small boxes out of which the pigeons had to take a certain number of peas. Occasionally the birds, together with the experimenter, 'invented' a new method. When the task was to open 2 boxes (out of 3) and to eat 2 peas, Arndt always put 1 pea in each of 2 of the boxes whilst the third box remained empty. But the question remained: was this a training to boxes or to peas? So he tried putting the second pea in box no. 3, and the pigeon opened all 3 boxes and ate 2 peas! Thus a new rather fruitful method was found: to open x boxes until 2 peas were obtained. This was later extended to the problem of taking 3, 4, 5, 6, and 7 peas out of a changing number of boxes. The more peas, the more possibilities of variation in placing them—5 peas out of 5 boxes give several hundreds of possibilities. Again 6 was the limit; 7 peas out of 7 boxes gave no reliable result.

Another new technique used by Arndt was the so-called 'premature frightening', e.g. birds fully trained to eat 4 grains were frightened away after they had only eaten 1, 2 or 3 grains. But in the next experiments the pigeons again ate 4 grains. This fact, according to Arndt, seems to prove that there is no 'inner counting' by the birds. In my opinion the author attaches too much importance to this fact, as we shall see later that Marold's budgerigars and Schiemann's jackdaws behaved in a different way under the same experimental conditions.

Arndt also tried to train his pigeons to perform two or three different tasks simultaneously. Two birds learned to eat either 2 or 4 (in later experiments 2 or 5) grains on bases of different colours, e.g. to eat 2 grains from a black and 4 (or later 5) from a white board. Another bird learned to take 4 grains out of boxes with white lids and only 2 grains out of boxes with yellow lids with a black line across them. It is significant that with simple white and black covers, or white and yellow covers, it was impossible to achieve the same result. Fischel's (1926) pigeons had not learnt to discriminate between 2 or 3 dots on the covers of food boxes when they were presented as ... and .., but Arndt succeeded by means of a temporary figural help. He started with \therefore against .. and transformed the triangle gradually into 3 dots in one line ..., but here a punishing device was necessary.

Finally every figural help was destroyed by offering successively either lids of boxes on a turntable, or peas which rolled down a pipe or chute. In the same way every possible temporal rhythm could be destroyed by varying the time intervals between 1 and 60 sec.—thus eliminating Bierens de Haan's criticism. Even under these conditions three pigeons were able to limit their pea-eating reaction to $x = 2$ to 5 and this is a very important

result. During this training an especially effective punishment was necessary (the ordinary springboard enlarged by long paper fans) as the birds could not otherwise 'resist the temptation' of pecking at a moving grain, an observation fully confirmed by Benner (1938) and Honigmann (1942*a*). When the training was finished the birds used to leave the place quickly before the first forbidden pea appeared.

In conclusion, Arndt stresses two important facts. (1) The observation repeatedly made how strongly the birds are accustomed to all the constant details of the apparatus although the 'decisive' conditions of the experiment (e.g. figural position, rhythm) were always changing. A minute area newly painted, a dash of chalk on the floor, or a passing woodlouse at once prevented the birds from working satisfactorily. (2) The second observation deals with the problem of learning without punishment—whether the 'disappointment' of finding no food would be sufficient to prevent the bird from opening empty boxes. Strictly speaking the 'disappointment' (there are no inverted commas in the original paper!) is a punishment, too, though a very mild one. The author himself stresses the fact that this method gave the best learning results. On the other hand some of Arndt's achievements were certainly only made possible with the help of a sharp punishment.

Marold (1939), continuing Arndt's work, used budgerigars (*Melopsittacus undulatus*) as subjects. He had hoped very much from their mental versatility but was disappointed as they were easily distracted by every external stimulus. On the other hand the birds were very persistent and many experiments could be performed without their becoming tired. In my opinion the adaptability and intelligence of parrots and parrakeets, except perhaps the African grey parrot (*Psittacus erithacus*), is usually greatly overestimated and far inferior to the abilities of most of the Corvidae.

Marold again examined the ability to repeat an action x times, and here, too, $x=6$ was the limit—a highly interesting result. Even birds which had not been previously trained in other tasks learned to eat 6 grains from a heap (usually of 10–15 grains). To achieve this more than 600 training experiments (with severe punishment in the case of a wrong choice) were necessary with a single bird, as the following table shows:

(-) = wrong number of grains. (+) = correct number of grains.

No. of experiments	Budgerigar's pecks						
	(-) 1	(-) 2	(-) 3	(-) 4	(-) 5	(+) 6	(-) > 6 grains
1-100	—	—	—	—	—	1	99 times
101-200	—	—	1	6	3	4	86 "
201-300	—	—	1	7	17	32	43 "
301-400	—	—	—	5	24	32	39 "
401-500	1	—	1	4	20	42	32 "
501-600	—	—	—	4	27	39	30 "
601-700	—	—	—	—	18	57	25 "

Thus the first success was visible during the seventh hundred (Exps. 601–700) and here still 43 % mistakes were made. But in 25 spontaneous experiments (without punishment), which could only be interposed one at a time, the result was:

No. of grains eaten	(-) 5	(+) 6	(-) 7	(-) 8	(-) 9
How often eaten	2	12	9	1	1

thus numerically confirming and safeguarding the previous results.

Training to eat 7 grains from a heap was a complete failure. The birds reacted by keeping above 5 and under 9, but this was all.

Marold succeeded for the first time in training a bird—actually it was only one of his 6 birds—to a triple task. He used three different signals to indicate the requirements, namely, (1) a black cardboard with chequered border for eating 2 grains, (2) a plain black one for 3 grains, and (3) a black one with yellow border for 4 grains. This success is an important achievement, as Sadovinkova's experiments (see p. 321) were only concerned with spatial and not with temporal relations. In addition, while in Sadovinkova's experiments the possibility of a technical error is not at all excluded, here all necessary precautions were taken. Marold did not dare to go further and give four or five tasks simultaneously. But he tried to combine both 'basic abilities' (p. 330) by using painted dots as indicators in double tasks, but here he was not successful at all (see Schiemann's experiments below).

Rather a large part of the paper is devoted to the problem of 'premature punishment'. Marold's results are different from those obtained by Arndt and he is at pains to explain this fact, as it does not agree with Koehler's conception, which must, to say the least, be expanded. The budgerigars behaved at first exactly like Arndt's pigeons, which means they did not take any notice at what point in their action ('Handlungsablauf') the premature punishment took place. But Marold tried two new ways: he used birds which had eaten amply before, and there was now a difference. This was not quite convincing, however, because satisfied birds, even without premature punishment, often take less than hungry ones. Marold therefore tried another method: he offered the grains in the test following the 'premature frightening' on a new board of an unusual form or colour, and then it was effective: the bird took only the number (or nearly the number) of grains it had eaten in the previous experiment. It was of course quite impossible to repeat these experiments too often, as otherwise it would have been a training for multiple tasks which was not intended, for Marold wanted to find out whether the bird would be able to 'keep in its mind' the point where it had been punished prematurely. This is of course highly interesting from a theoretical point of view, and in the case of the budgerigars it succeeded to a certain degree, provided a new visual stimulus was added, distracting the bird from its 'usual automatism'. One can keep Koehler's original conception by attributing different valences (E. S. Russell) to the different grains. The valence of the first grain is maximal, the valences of the following grains decrease, and so on. Therefore in a training to high numbers it would be a mistake to punish as severely as in a training to low numbers, otherwise the bird would take too little.

While Marold did not attempt to investigate the upper limit of 'basic ability no. 1' (discrimination between 4 and 3, 5 and 4, etc.), he was able to train budgerigars to distinguish 2 dots from 3 dots on lids of food boxes, without even 'figural help'. He stresses the fact that here the primary and the secondary valences of the groups must be considered, for it is easier to train to the larger group, as here the primary valence (more food) works in the same direction as the secondary valence produced by the training. In training to the smaller group, however, the primary valence is opposed to the secondary one, but can be suppressed by suitable measures. Actually such a training is very difficult when the task is as simple as discriminating between 2 and 3.

Schiemann (1940) investigated the two 'basic abilities' of jackdaws (*Corvus monedula*), and although he used nearly the same methods as his predecessors his paper reveals a

number of highly interesting new facts. In contrast to the domesticated birds used previously (pigeons, budgerigars) punishment proved here to be much more detrimental, particularly in unsolvable tasks. The limit in repeating an action was again 6, as it was before with other birds, and the training to take 7 mealworms and then to stop was never achieved.

When the task was to open a varying number of dishes containing changing numbers of bait the limit was again 4 filled dishes, as with Arndt's pigeons. During these experiments Schiemann twice made a highly significant observation. One jackdaw, during a series of trials where the task was to take 5 mealworms out of 4 dishes, had been punished for having opened too many dishes. In the following two tests the bird took only 3 and 4 mealworms respectively, instead of the 5 allowed. During the next trial 6 dishes with 5 mealworms were used in the following way: ① ① ① ① ① ①, i.e. dish

no. 2 contained 2 mealworms, dishes 4 and 6 were empty and the rest contained 1 mealworm each. The jackdaw, still under the influence of the previous punishment, opened only the first 4 dishes and thus obtained only 4 mealworms instead of the 5 permitted. Then the bird, after looking at the punishing device, went back to the door and started again: it nodded once at dish no. 1 (open and empty), nodded twice at dish no. 2, again once at dish no. 3. Then, after a short pause, and without taking any notice of dish no. 4, the bird opened dish no. 5 and took the missing mealworm. The author says that the bird, as it were, repeated its action with 'intentional movements', and only in the case of the still closed dish no. 5 actually performed the pecking. Such behaviour is like that of a child stuck while reciting a poem, who has to start again from the very beginning to overcome the difficulty.

Experiments with 'premature punishment' confirmed Marold's experience with parakeets, but with the jackdaws there was a distinct influence even without any modification of the usual experimental conditions. When birds trained to take x objects were punished after having taken only y ($y < x$), they afterwards kept on taking only y with considerable accuracy up to a maximum of 28 tests (lasting 35 min.) without punishment. Nevertheless Schiemann did not abandon the original conception of Koehler, and he agrees with Marold's explanation in spite of the difference in results.

In addition the author made some experiments with human beings, for example his brother aged 16. The boy had to knock on the table x times with a spoon while doing some mental arithmetic, being thus prevented from counting the knocks. The limit was again 6 as in the case of the birds:

$x =$	Correct responses %	Error limit
4	98	4-5 knocks
5	62	4-7 "
6	56	4-8 "
7	27	5-10 "

In the case of knocking 7 times there was far less than 50% of right solutions, and when $x > 7$ hardly any. By punishing his brother prematurely (and unexpectedly) with a severe electric shock Schiemann found that there was no real counting in these tests. Actually the shock was administered with the fifth knock, but the 'human guinea-pig' believed it was given after the third one.

Schiemann did not repeat Arndt's experiments of offering various objects successively,

but in the case of multiple tasks he was able to beat all previous records with his jackdaws. When different colours were used as signals or indicators these birds could perform 4 different tasks simultaneously, e.g. to take 2, 3, 4 or 5 specimens of bait. For obvious reasons Schiemann did not attempt to extend this experiment to the task of selecting 1 or 6 pieces of bait.

'Choice from sample' was achieved with colours and groups of dots on the lids. One jackdaw judged the dots only as figures, but another one according to the numbers of dots, independent of the position and size of the dots, although this success did not last. A third jackdaw combined both basic abilities by accepting a 2-dot lid as indicator for eating 2 pieces of food and a 4-dot lid as indicator for eating 4. Here some figural help was necessary in the beginning, but later a 'certain independence of it' was achieved. These experiments prove clearly that for the animal there are no primary relations between the 2 and 4 dots and the 2 or 4 pieces of food allowed. For human beings this would be very simple, whereas the birds had to learn it laboriously. On the other hand we should certainly have more difficulty in learning that one colour means a certain number of objects and another colour a different number. This, however, is a relatively easy task for the bird, while the co-ordination between the observed numbers of dots and the response in eating the same number of peas is the limit of the bird's learning ability.

In nearly all these tasks the jackdaws achieved more than either pigeons or parakeets. Increasing difficulties had the effect that they learned quicker and quicker to retain facts experienced only once with all their details in their memory, and to reproduce them accordingly in their reactions. But even these highly adaptable birds, as we have seen, are entirely unable to 'count' in the human sense of the word.

VI. SUMMARY

1. The first attempts to approach the problem of number conception in animals consisted in a training to identify one object out of many by its relative position.

2. The multiple-choice method, although an advance on previous work, has yielded relatively poor results. The only apparent exception, namely the striking results obtained with finches, is probably due to a technical error.

3. Experiments with a temporal maze have shown that it is extremely difficult for animals to perform a double alternation, and no animal has yet been trained to perform this more than once successively. Claims that some birds are able to select every third out of a line of similar objects are without convincing foundation owing to the lack of adequate control tests.

4. The experiments of Verlaine and his collaborators led to the belief in a genuine number conception in animals, but the lack of satisfactory controls deprives the results of these cleverly devised experiments of validity.

5. Bierens de Haan repeated these experiments on a sound basis with the necessary controls. He found that the animals trained themselves to perform two or three single actions in a certain rhythm, and that consequently increasing the intervals between the single actions destroyed the training.

6. Systematic investigation of the two basic abilities of performing an action a certain number of times and of discriminating between two quantities of objects gave the interesting result that the limit in both cases is respectively 6 or 5:6 for all animals investigated. The discrimination between 6 and 7 has never been achieved.

7. Experiments with parakeets gave better results than with pigeons, but both groups were surpassed by the achievements of jackdaws. The latter were able to learn four different kinds of number training, and to make use of them simultaneously. They could also to a certain extent combine both basic abilities. But a close analysis of these surprising accomplishments shows that even here any counting or number conception in the human sense of the word does not exist.

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